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SOME PROBLEMS IN THE PATHOLOGIC HISTOLOGY OF CARCINOMA OF CERVIX UTERI, WITH SPECIAL CONSIDERATION ON ITS RELATIONSHIP TO PROGNOSIS.
(With Plates XV and XVI)

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PREFACE

One of the greatest problems in the field of gynecology is that of carcinoma of uterus. As is well known, in 1950 the Gynecological Society of the United States of America presented a classification of carcinoma of cervix uteri recommending as a new international classification, which divides them into five stages, namely, from O to IV. However, such a clinical classification cannot yet be called complete without fundamental histologic examinations sufficient for it—such as investigation on the nature of O stage, namely carcinoma in situ, and also on the mode of infiltration in the parametrium, which is problematical concerning the II and III stages.

As early as 1920, also in U.S.A., Broders divided carcinoma, particularly epidermoid carcinoma, histologically into four grades and he suggested a possibility to forecast prognosis from the histologic structure of tumor. Ever since many clinical pathologists in U. S. A. have shown much interest in the histologic grading and also many clinicians have made attempts to judge prognosis on the basis of such grading.

On the other hand, there are not a few who are rather critical on such a view that affirms the possibility of judgment on the prognosis of carcinoma simply from the histologic structure. For instance, Rössle (1950), a noted German pathologist, and Ogata, Yoshida, etc., of our country are not always in favor of this view.

In the meanwhile, Imai (1950) has recently suggested what he called C. P. L. classification, a new one concerning carcinomatous development based on a detailed investigation on the histologic structure of carcinoma, taking not only the development of parenchyma but also the reaction of stroma into consideration. He and his co-workers, Matsumoto, Tanaka, Oka, and Okamoto studied on gastric, mammary, laryngeal, and uterine carcinomas using large specimens and asserted that the prognostic judgement based on his classification is quite reliable.

What I attempt here is to make a summary report on the result of my investigation concerning the above problem based on the material collected in our department, such as autopsy, surgically exstirpated or biopsy specimens, vaginal smears, etc.,

of uterine carcinomas. Concerning the problem of prognosis, exceedingly valuable for my investigation were the 278 cases of carcinomas of cervix uteri, on which Dr. Kyusaku Ogino of Takeyama Hospital in Niigata City operated by Okabayashi's method of extended hysterectomy modified by Ogino and the prognosis of which he also pursued for five years or more after operation. However, since this clinical material lacks in post mortem examination, I have made up for this deficiency by investigating the 35 autopsy cases of carcinomas of cervix uteri, 27 of which issued from our department and 8 kindly offered by the Pathological Department of Fukushima Medical College.

The Problem Concerning Histologic Figure and Clinical Malignancy of the Carcinoma of Cervix Uteri.

A) Material.

The material of our research work is as was mentioned above ; the above 278 carcinomas of cervix uteri, examined in connection with prognostic judgement are divided, according to the international classification, into I stage 49, II stage 192, and III stage 36. On the other hand, 33 of them are of adenocarcinoma and carcinoma simplex types, and 245 are of spindle cell cancer, spinal cell cancer and intermediate types (so-called transitional cell cancer). 3 patients out of the former group and 25 out of the latter either expired at hospital after operation or died of complications such as bacillary dysentery, tuberculosis, cerebral apoplexia, etc., after leaving the hospital and consequently these 28 cases were eliminated from the group for prognostic judgment. Adenocarcinomas always develop from the glandular epithelium, but their figures widely vary, some showing distinct structure of gland, others that of papillary adenocarcinoma, and still others transitional figures toward carcinoma simplex. So it involves inconsistency in itself, to divide them into 4 stages difinitely as Broders did.

What come under the category of epidermoid carcinomas are characterized with the marked variety of the structure, as has been known. Not a few of them show different structure according to portions even in one and the same case. The histological classification I attempt to make as objectively as possible is as fallows :

Classification of tumors	Number of cases
I. Epidermoid carcinoma group.	(245)
A) Spindle cell cancer and type dominated by such.	(93)
1) Spindle cell cancer.	(23)
2) Chiefly spindle cell cancer, but partially transitional cell cancer or spinal cell cancer.	(51)
3) Chiefly spindle cell cancer but partially transitional cell cancer and spinal cell cancer.	(19)
B) Transitional cell cancer and type dominated by such.	(74)
1) Transitional cell cancer.	(28)
2) Chiefly transitional cell cancer, but partially spindle cell cancer or	

spinal cell cancer. (39)

3) Chiefly transitional cell cancer, but partially spindle cell cancer and spinal cell cancer. (7)

C) Spinal cell cancer or types dominated by such. (78)

1) Non-cornificated spinal cell cancer and type dominated by such. (33)

i) Non-cornificated spinal cell cancer. (12)

ii) Chiefly non-cornificated spinal cell cancer but partially spindle cell cancer or transitional cell cancer (20)

iii) Chiefly non-cornificated spinal cell cancer but partially spindle cell cancer and spinal cell cancer. (1)

2) Cornificated spinal cell cancer (cancroid) and types dominated by such. (45)

i) Cancroid. (21)

ii) Chiefly cancroid, but partially spindle cell cancer and transitional cell cancer and transitional cell cancer. (19)

II. Adenocarcinoma group. (33)

A) Adenocarcinoma and type dominated by such (23)

1) Adenocarcinoma (partially papillary adenocarcinoma). (4)

2) Chiefly adenocarcinoma but partially carcinoma simplex, spindle cell cancer, transitional cell cancer or epidermoid carcinoma. (19)

B) Carcinoma simplex (including so-called diffusive infiltration type) or type dominated by such. (10)

B) The limit of recognition of cytologic or histomorphologic characteristics of cancer cells.

Although the causal factor of human cancer is still in the dark for the greater part, it is generally agreed that the normal epithelial cells proper to human body turn carcinomatous by the activity of some carcinogenic factors. Today the most authentic explanation of the mechanism for the acquisition of such peculiar nature by the normal cells that shows limitless autonomous proliferation as carcinoma is the theory of mutation. It should be remembered, however, that none have yet grasped this phenomenon as a purely objective fact on the cellular or nuclear structure, and this theory is also but an assumption after all.

At any rate, in the cells once turned carcinomatous various aspects in the cytologic structure differing from that of normal cells, their matrix, are discernible. For instance, deep staining of the nuclei, general enlargement of nucleus in comparison with the cell body, frequent presence of mitotic figures, more or less basophil staining of the cytoplasm, etc., are tendencies commonly known. Besides, in the so-called atypical carcinoma, both the nuclei and the cell bodies are irregular in form and unequal in size, often showing atypical mitosis. Thus between normal cells and carcinoma cells many points of distinction are detectable, which make it possible, to a certain extent, to identify carcinomatous or malignant tumor cells by observing some individual cells. Here lies the theoretical ground for the attempt such as the Papanicolaou method, by which a diagnosis of uterine cancer can be made from the appearance of vaginal smears.

However, such metamorphosis of various epithelial cells is not always peculiar to carcinoma. I myself have a bitter experience of misjudging, referring to the matter: on a case with persistent hematuria from the exsistence of seemingly malignant cells in the catheter urine a diagnosis of renal carcinoma was made by me and an operation was advised. The postoperative pathologic examination, however, revealed only an atypical proliferation of the epithelium of the renal pelvis (so-called precancerous change). Again recently I judged similar cells appearing in groups in a vaginal smear as carcinoma cells, and as the result of examination of curettage material, they were found to be cells distorted by some inflammatory process seen in a part of the endometrium. Thus occasionally the cellular metamorphosis which is considered to be peculiar to cancer can occur in inflammations. So the most experienced pathologist, even with the most excellent preparations, may be erroneous, if diagnosis of uterine cancer is depended solely on the Papanicolaou method. Therefore the most important problem for clinicians as well as pathologists is to find some way to distinguish carcinomatous cell transformation from precancerous change; consequently the problem which gives us most attention in the inquiry into the carcinoma of cervix uteri is that of carcinoma in situ. There are varied interpretations for this name, which must be due to the difficulty in differentiating carcinoma from precancerous change or such non-cancerous epithelial proliferation as caused by inflammatory stimulation. Carcinoma in situ literally means the carcinoma within the epithelial layer, namely, that with no infiltration through the basement membrane (Broders, Schiller, Hoffman). And it involves the precancerous state since the histologic differentiation between carcinoma and precancerous change is so difficult. However, it may be adequate to eliminate such a state that will fade away without indicating downgrowth from under the category of carcinoma in situ.

Such being the present state of investigation, most earnest efforts have been made in order to grasp the real characteristics of cancer cells by means of the studies on chromosomen or with the aid of recently improved histochemical methods of investigation of nucleic acids (Casperson and his collaborators, Cusmano, etc.). Casperson and his collaborators state, from the result of color reactions of nucleic acids, that in carcinoma are discerned two types of cells, namely A type with abundant and B type with scanty amount of D. N. A. in comparison to normal cells. And they regard the former as active cancer cells in the state of fission and proliferation and the latter as those in regressive degeneration According to Usuda, also, both D. N. A. and R. N. A. are generally found increasing in the carcinoma cells of cervix uteri, but the increase is not at all peculiar to tumor cells, being rather common finding to the cells with marked tendency of proliferation. The investigators assert that the cells in degenerating or stastic stage (which Casperson, etc., call B type) the amount of nucleic acids either has ceased to in-

crease or decreases. So in the present stage of our knowledge any satisfactory histologic or cytologic differentiation between cells in precancerous change and malignant tumor cells cannot be expected by means of the investigation of nucleic acids. In short, the differentiation between the cells in precancerous process and those turned cancerous by the so-called mutation is not yet possible.

Then it has been a common sense in pathology that carcinoma cells infiltrate into the deep stroma through the basement membrane and the latter is not found around carcinoma cell nest. Borst (1924), a famous oncologist, mentioned this clearly in the chapter of malignant tumor in his "Geschwulstlehre", which has been believed without further re-examination. However, even the histologic elucidation concerning basemnent membrane itself has been insufficient and Muto (1937) pointed out intensively that Borst's view is not correct. Muto and his coworkers have published many excellent works in this line of study and the result of my research work agrees with theirs completely. Namely, according to both Muto et al. and I, what is called basement membrane is no other than a kind of argyrophil fiber network found in the portions where collagenous fibrs get in contact with epithelial cell layer, capillary wall, muscular fiber bundle, etc., and close transitional figures between the said membrane and the collagenous fibers are discernible. Muto et al., on inserting various kinds of artificial fibers into the subcutaneous tissue of animals, have ascertained the fact that, according to the form and nature of the inserted fibers, some of them cause the formation of basement membrane beautifully around them but others do not or cause it quite meagerly. So it may be understood that it is not right to regard basement membrane as an fiber network entirely different from collegenous fibers. It is rather more probable that some collagenous fibers themselves turn to be silver impregnable fibers under such environmental changes as the insertion of foreign substance (artificial fibers) or the infiltration of inflammatory cells. Muto (1937) also verified histologically that sometimes lacerations are seen in the subcutaneous basement membrane; and so it is easily understood that the cancer cells newly grown in the epithelial cell layer possibly proliferate into the deep layer—so-called cancerous downgrowth—through such openings in the basement membrane. If so, it may not be wrong to regard such a phenomenon as a histologic characteristic of carcinoma. However, we occasionally meet with such a uterine cancer, in which carcinoma cells proliferate only along the surface instead of permeating the deep layer (for example, the cancer of the portio vaginalis develops along the mucosal surface of the cervix uteri or along the inner surface of vagina). Also under some circumstances, even in the carcinoma with downgrowth interstitial connective tissue may develop a basement membrane in contact with carcinoma cell nest. So we can hardly agree with those who try to draw a definite line of differentiation between cancer and carcinoma in situ using this downgrowth as the criterion, even though the tumorous parenchymal cells of the latter have

not yet permeated through the basement membrane.

C) A Comment on the Past Histologic Classification of the Malignancy of Uterine Carcinoma

In Henke-Lubarsch's Handbook of Pathology, R. Meyer states, as if the matter were difinitely settled, that the carcinoma of cervix uteri can be classified into several stages of ripe and unripe types from its histologic structure regardless of whether it is adenocarcinoma or epidermoid carcinoma. In regard to adenocarcinoma group a typical adenomatous structure is characteristic of the ripe type, whereas what is called carcinoma solidum or carcinoma simplex is less ripe, and further, the type which diffuse infiltration is least ripe. The same can be said concerning the relation between spinal cell cancer and spindle cell cancer, the former being ripe, the latter less ripe. Further, some authros interpose one or two stages between the two types. The criterion for such differentiation between the ripe and unripe types is, in epidermoid carcinoma, for instance, that the spindle cells are physiologically younger than or in the stage prior to the squamous cells. Again, as for adenocarcinoma group, carcinoma simplex is regarded less ripe than adenocarcinoma, probably because the adenomatous structure is embryologically differentiated from the solid epithelial cell sheet. Whereas we admit that solid cell group and spindle cell group are younger than glandular or squamous epithelium, in developmental stage there has been made hardly any investigation sufficient to convince us of the same variance in the degree of malignancy between the two. Presumably this view has its ground in the experience that in human body what is called the unripe type develops faster, or more frequently makes metastasis than the ripe type. It is not too much to say that such a merely imaginative factor has been the basis of the past conception of the degree of ripeness of carcinoma. In my research work, I encountered not infrequently histologic pictures, in which a part of adenocarcinoma turns to carcinoma simplex, or diffuse type and spinal cell cancer in some portions take the form of spindle cell cancer or transitional cell cancer, or these histologic types are mixed. According to the past interpretation these phenomena mean that a carcinoma originated in a human body changes its degree of maturity in its course of growth. In the process of cancerous development retrogressive metamorphosis, namely, turning to less matured types can easily be imaginable, but there are some cases in my material, in which the primary focus shows a figure of spindle cell cancer but the metastasis of some lymph nodes does show that of spinal cell cancer. If we follow the above assumption we have to admit the possibility for carcinoma to turn more benign. Yoshida, on the contrary, on the basis of his experiments on Yoshida sarcoma, which has been experimentally produced by him and been named after him, states that this sarcoma never changes its degree of malignancy, regardless of how often it may be transplanted or in what stage of its growth it may be transplanted. Whether or not such a fact in rat sarcoma is immediately

applicable to human cancer may be a question, but it sounds unbelievable that the carcinoma cells originated in one and the same organism should grow unripe and malignant in some portions while they remain ripe and benign in others. Nevertheless, I do not mean that carcinoma should always maintain the same degree of malignancy. Although it can hardly be expected in human body that the carcinoma losing its malignancy, should show natural regression, it may be possible that carcinoma should increase its degree of malignancy. By what mechanism does the aggraviation (getting more malignant) of the tumor cells occur? More probable than the conception of gradual changes is that of mutation, by which the tumor cells change their nature suddenly, just as the cells in precancerous state grow carcinomatous at a certain time by mutation (Yoshida). This suggests considerable inconsistency in the idea of determining the degree of malignancy of carcinoma cells by their histologic types. However, I will first try to make a statistic examination on my material according to conventional theories. Among many classification principles, I will choose here those of Broders and Martzloff, two of the most prevailing in U.S.A.

1) Discussion on Broders' classification.

As is well known Broders divides the degree of malignancy of carcinoma into following 4 stages according to the numerical ratio between the differentiated and undifferentiated types of carcinoma cells.

Grade I : The carcinoma with undifferentisted cells below 25% and differentiated cells above 75%.

Grade II : The carcinoma with 25% to 50% undifferentiated cells, the remainder being differentiated cells.

Grade III: The carcinoma with 50% to 75% undifferentiated cells, the remainder being differentiated cells.

Grade IV: The carcinoma with undifferentiated cells over 75%, the remainder being differentiated cells.

As the characteristics of such undifferentiated carcinoma cells he mentions the presence of one large deeply stainable nucleolus, the existence of mitotic figure, and the irregularity of nuclear structure. Is it possible, however, to grade carcinoma objectively, if one be obedient to Broders' description with illustrations? As was stated before, since it is considered to be impossible even to differentiate the cells in precancerous state from the cancer cells, it is rather risky to differentiate the riper cells from the unripe among the carcinoma cells. However, in U.S.A. this carcinoma grading of malignancy by Broders made public in 1920 seems to be still most widely used, although there are not a few who would not approve of it.

Martzloff, of whom I will state in the following paragraph, regarde his Grades I, II and III corresponding to Broders' Grades II, III, and IV, respectively. From this fact we know that Broders' classification is not always used without alteration. Such being the circumstance, I gave up my plan to investigate on my material

according to Broders' classification.

2) Discussion on Martzloff's classification.

Martzloff divided carcinoma of cervix uteri originated in the covering squamous epithelium into i) spindle cell cancer, ii) transitional cell cancer or intermediate type, and iii) spinal cell cancer. What he calls here the intermediate type is the carcinoma, whose cells are neither of spindle cell shape nor of typical spinal cell form but are in transitional aspect between the two cell types, mostly with indistinct cell boundary. According to this classification, the 245 cases of my material can be classified, as was mentioned above, under (1) spindle cell cancer 93, (2) transitional cell cancer 74, and (3) spinal cell cancer 78. Among these cases are eliminated 11 cases of spindle cell cancer, 4 or transitional cell cancer, and 10 of spinal cell cancer, in which death occurred at hospital or after leaving the hospital from other diseases than carcinoma. The following table shows the prognosis of the 220 cases.

	Healthy	Death 5 yrs. afterwards	Death within 5 yrs.	Total of death cases	Total cases
Spindle cell cancer	59 (71.9%)	2 (2.5%)	21 (25.6%)	23 (28.1%)	82
Transitional cell cancer	44 (62.8%)	2 (3.6%)	24 (34.2%)	26 (37.2%)	70
Spinal cell cancer	53 (77.9%)	1 (1.6%)	14 (20.5%)	15 (22.1%)	68
Total (%)	156 (70.9%)	5 (2.3%)	59 (26.8%)	64 (29.1%)	220

Usually in clinicians' statistics the cases of death 5 years after leaving the hospital is included in the column of "healthy" but I tried to show the two separately. The percentage of healthy cases to the total number, no matter whether the cases of death 5 years afterwards be included in the healthy cases or in the death cases, is highest in spinal cell cancer group, and lowest in transitional cell cancer group. But stochastically (estimated by χ^2 test) there is no significant difference among these groups. So there is obviously no foundation for Martzloff's idea to estimate the malignacy of carcinoma of cervix uteri, especially epidermoid cancer from the histologic type and also for the vague, conventional conception that spindel cell cancer is more undifferentiated and more malignant than spinal cell cancer.

(Note) The two types of adenocarcinoma group originated in glandular epithelium, namely cancer with adenomatous structure and carcinoma simplex, entirely or dominantly, are compared in the following table as to their prognosis, in the same way as the above table of epidermoid cancer group. Here also the difference of prognosis between the two types is not significant stochastically.

	Healthy	Death 5 yrs. afterwards	Death within 5 yrs.	Total of death cases	Total cases
Carcinoma simplex	4 (50.0%)	0 (0%)	4 (50.0%)	4 (50.0%)	8
Adeno-carcinoma	12 (54.5%)	0 (0%)	10 (45.5%)	10 (45.5%)	22
Total	16 (53.3%)	0 (0%)	14 (46.7%)	14 (46.7%)	30

3) **The relationship between mitosis of cancer cells and prognosis.**

I do not need to explain here that mitotic figure of the nucleus is a barometer indicating rapid cell proliferation, but most serious caution must be taken in making any comparison of the figure versus the malignancy of neoplastic cells. It is not only because my research specimens are different in the time lapsed from operation to fixation, but also because it is inferable that the tempo of cell division of tumor may not be constant but undulate. This is evident from the fact that in spindle cell cancers, for instance, the number of mitotic figures is extremely varied from case to case and even in one and the same case, the number often varies according to the portion of development. As was stated above, it has come to be clear that to discuss the prognosis by dividing carcinoma of cervix uteri into epidermoid carcinoma and adenocarcinoma is not worth attempting. Therefore, I have tried to discuss them as a whole. I have calculated the number of mitosis in one range at 280 χ magnification of Zeiss' microscope and marked below 5, from 6 to 10, and above 11 mitoses as +, ++, and +++, respectively. Thus the relation between the numerical grade of mitosis and prognosis is shown in the following table.

	Healthy	Death 5 yrs. afterwards	Death within 5 yrs.	Total of death cases	Total cases
+	102 (69.3%)	3 (2.2%)	42 (28.5%)	45 (30.7%)	147
++	47 (70.1%)	1 (1.6%)	19 (28.3%)	20 (29.9%)	67
+++	23 (63.8%)	1 (2.9%)	12 (33.3%)	13 (36.2%)	36
Total	172 (68.8%)	5 (2.0%)	73 (29.2%)	78 (31.2%)	250

As it is evident from the above table, no distict relation was proved between mitosis and pronosis.

4) **The C. P. L. classification of developmental form of carcinomas of cervix uteri, related with their prognosis.**

Recently Imai (1951) as the result of his comprehensive investigation with not only the developmental form of cancerous parenchyma but also the stromal reaction to it in consideration, divided the developmental forms of carcinoma into 3 types namely ; C type (abbreviation of cirrhotic form), P type (abr. of progressive form) and L type (abr. of lymphatic form). He points out the fact that the C. P. L. classification based on the figure of carcinomatous development in the perifocal portion of the carcinoma (where a new developmental expansion is seen) well serves to suggest the prognosis. He stresses that in the organ itself where the carcinoma has originated, the so-called anti-carcinomatous stromal reaction occurs instead of letting the cancer cells freely prey upon. As the histologic signs of this phenomenon he mentions the reaction by various wandering and fixed cells and the proliferation of the connective tissue that follows it. He calls this form with cellular reaction and stromal connective tissue proliferation C type. Further, those in which connective tissue proliferation and inflammatory cell reaction are not or slightly seen in the perifocal stroma (Imai calls such a carcinomatous focus "cancerous lump") he devides into 2 types, namely, the progressive (P type) and lymphatic (L type) types. The former is the type in which carcinomatous cells infiltrate into the pre-existing tissue spaces and develop there, and the latter is the type in which cancer cell groups invade into the comparatively large lymphatic vessels. According to him these are both non-reactive carcinomatous proliferation, which he calls the "Schub" of cancerous development as compared to the tuberculous process. He notices a close relationship between P type and L type development, and divides the two into 3 grades namely, grade I (P I, L I) grade II (P II, L II) and grade III (P III, L III). He states that the grade III in both types is suggestive of very hopeless prognosis, which is always evident from autopsy material ; whereas C type usually predicts hopeful prognosis. I found the result of investigation upon my material according to Imai's C. P. L. classification satisfactorily agree with his view. I will briefly show the result in a table and try a stochastic examination. The epidermoid group and adenocarcinoma group, whether they are treated with separately or en bloc, show the same result. So I will give here only the table concerning carcinoma of cervix uteri as a whole.

The relation of L type cancer development with the prognosis: From this table it is quite evident that the degree of cancer cell invasion in the lymphatic vessels is in closs correspondence with the prognosis. Only in one case of spindle cell cancer which shows L III in the table the patient has been healthy over 6 years after operation. This case was clinically in the stage II of carcinoma of cervix uteri, in whose lymph nodes a large number of metastatic foci were present. Presumably these happened to be completely eliminated by the operation, the surgical attack having been given very shortly after what Imai calls the Schub. This is a case

which best shows the excellence of Okabayashi's extended hysterectomy modified by Ogino.

	Healthy	Death 5 yrs. afterwards	Death within 5 yrs.	Total of death cases	Total cases
L O	119 (85.0%)	3 (2.2%)	18 (12.8%)	21 (15.0%)	140
L I	38 (64.4%)	3 (5.1%)	18 (30.5%)	21 (35.5%)	59
L II	14 (41.1%)	0 (0%)	20 (58.9%)	20 (58.9%)	34
L III	1 (5.9%)	0 (0%)	16 (94.1%)	16 (94.1%)	17

L O = No cancer cell invasion in lymphatic vessels.
L I = Occasisional invasion of cancer cells in lymphatic vessels.
L II = Intermediate degree between L I and L III.
L III = Frequent invasion of cancer cells in lymphatic vessels.

The relation of P type cancer development with the prognosis: By P type cancer development here I do not mean purely progressive development itself but I mean the P type element seen in the form of perifocal cancer development. Namely, P III type is the developmental condition in which the cancer cells without cellular or connective tissue reaction are invading in the tissue spaces in the greater part of the range of microscopic vision; P I type is that which shows mostly C type development with occasional mixture of P type. P II is the intermediate type between these two types. Both P II and P III types frequently involve, as is naturally expected, the cancer cell invasion into the lymphatic vessels (L type), and combination type P III, L III is also often encountered.

	Healthy	Death 5 yrs. afterwards	Death within 5 yrs.	Total of death cases	Total cases
P I	30 (65.2%)	2 (4.4%)	14 (30.4%)	16 (34.8%)	46
P II	10 (71.4%)	2 (14.3%)	2 (14.3%)	4 (28.6%)	14
P III	2 (25.0%)	0 (0%)	6 (75.0%)	6 (75.0%)	8

As is evident from the table (the same can be said by stochastic examination, of course) the advanced progressive type (PIII type) is very hopeless.

The relation of C type cancer depelopment with the prognosis: What I call here the C type cancer development according to Imai's classification, of course, consists

mostly of pure, unmixed type, but it also includes kinds of chiefly C type but partly P I or L I type. The following table shows the fact that a greater part of the patients (86.5%) whose excised uterine cancer showed histologic figures of C type remained healthy at least for 5 years.

Healthy	Death 5 yrs. afterwards	Death within 5 yrs.	Total of death cases	Total cases
130 (83.8%)	4 (2.7%)	21 (13.5%)	25 (16.2%)	155

Discussion on the findings of autopsy material of carcinoma of cervix uteri: The material were 27 autopsy cases in our department and 8 such cases offered by Fukushima Medical College. Out of these 35 cases I will discuss on 26 whose histologic figures of the primary cancer foci I could investigate on. As Imai has already pointed out, in perifocal portions of these cancers L and P types, especially L III type, P III type, and L II, P II type are dominant, in which stromal connective tissue proliferation and cellular reaction are not or are seen only slightly. In other words, what Imai calls the Schub figures of cancer development are present in all these autopsy cases.

In the cases of uterine cancer with Imai's C type often show cellular infiltration, and the infiltrating cells widely vary in cases, including eosinophil and neutrophil leucocytes, sometimes in a surprisingly large amount. Since the foci of uterine cancer is originally exposed to bacterial infection, it is not strange that cellular infiltration should be found. Consequently I cannot approve of regarding all such cell infiltrations as Imai's anti-carcerous reactions. However, I do not hesitate to presume that what Imai calls anti-cancerous reactions are included in the above cellular infiltration, because in the above mentioned autopsy cases P and L types of development are exclusively discerned and the cellular infiltration was as a rule slight, where a new developmental proliferation of the cancer cells is going on; and also because the result of the histologic investigation concerning tumor immunity of the cancer-transplanted animals speaks for this.

The applicability of C.P.L. classification (Imai's) and its defense mechanism: As was stated before, Imai's C. P. L. classification based on the variance of the condition of histologic figures (both of parenchyma and stroma) in the cancer development terminals predicts the prognosis of the patients with considerable accuracy. If a large number of cancer cells are found invading into the lymphatic vessles of the operatively excised uterus, the prognosis will be hopeless with the probability of more than 90%, and as will be state later, with biopsy tissues the same is also sometimes true. The prognosis of C type is very hopeful just reverse to the above. Since both L type and typical C type are easy to be diagnosed histologically, the practical value of the said classification is great. As to P type, on the contrary,

although the judgments on such an evident figure as P III type may easily be brought to agreement, the determination of the criteria on P I and P II types is apt to be influenced by subjective judgment. It is fortunate that such a grading have not much significance in prognostic judgment. Imai's explanation for such variance in cancerous development types is, as was previously mentioned, that of the variance in vital reaction. That is, he takes the cellular infiltration in the cancerous tissue resulting in the connective tissue proliferation as the sufficient evidence of the anti-cancerous defence of the organism, whereas no or only weak cellular reaction or no connective tissue proliferation in spite of the cancer cell invasion into the tissue spaces or lymphatic vessel shows either the lack or insufficiency of anti-cancerous reaction in the organism.

My findings also support Imai's view that theae exists a close connection between the type with cancer cell invasion into the tissue space and that with further lymphatic vessel invasion. The problem lies in what the nature of such defence activity of normal tissue against the cancer cells is. That in the animals in which malignant tumor is transplanted some kind of anti-body is formed with the tumor cell themselves as the antigen, which makes the transplantation difficult; and that in such a case inflammatory cellular reaction occurs in the stroma has been verified by Takeda and his collaborators from the investigations on Yoshida sarcoma. However, this is a fact proved in the animals transplanted with the sarcoma from different strain of the animals; in those animals it may well occur that the tumor cells themselves as heterogeneous protein serve as the antigen. However, it must be different with human cancer. It is hardly believable that the cancerous cells should turn to protein so completely different as to be antigenic to the host, even if the carcinoma cells are cells changed by mutation from normal epithelium. Nor have we heard of the formation of immune bodies in human cancer. However, by admitting that the carcinomatous development is inhibited in the form of the cellular reaction or connective tissue proliferation in C type of development and that such a inhibitory activity is lacking in both L and P types, we can explain clearly the said relationship between the cancerous types and the prognosis. As was evident in my material, the mode of such reactions vary in each case, and even in one and the same carcinoma show extremely varied histologic figures, suggestive of the undulatory reactivity of the host to cancer development. Thus it is impossible fully to explain the nature of Imai's anti-cancerous reaction in human body by means of antigen-antibody reaction. At the present stage of our knowledge, there is no other way of explanation than to regard it as a kind of vital defence against the carcinomatous tissue. However, those pathologic achievements in this line of study coupled with the biochemical effort will bring about a clearer elucidation of the problem in the near future; for we hear that in the biochemical field the increase of such enzyme-inhibitory substances as anti-trypsin factor and hyaluro-

nidase-inhibitor in cancer patients have been discovered.

The relation between carcinomatous metastasis of lymph nodes and the prognosis: From the fact that cancer cell invasion into the lymphatic vessels has a close relation with the prognosis it is naturally inferable that the metastasis in the operatively excised lymph nodes must suggest something concerning the prognosis. The following table shows such lymph node metastases.

	Healthy	Death 5 yrs. afterwards	Death within 5 yrs.	Total cases of death	Total cases
Metastasis detected	27 (37.5%)	2 (2.8%)	43 (59.7%)	45 (62.5%)	72
No metastasis	145 (81.4%)	3 (1.7%)	30 (16.9%)	33 (18.6%)	178

As is evident in this table, the cases with lymph node metastases are markedly worse in prognosis than those without them; although some lymph node metastases may escape from my observation, for all the lymph nodes are not examined in serial sections. It is natural that in more enlarged lymph nodes, metastasis is more easily found, but as metastasis detected though rarely even in so small a node as miliary size, the observation should be made on very small ones. Seemingly it is more difficult to judge prognosis from the presence or absence of lymph node metastasis than from the existence of L III type in the primary focus of uterus. The reason for this is partly that is at the time of examination cancer cells may invade into the lymphatic vessels only and not yet into the lymph nodes, and partly that some lymph node metastases may escape from observation, as was mentioned above.

Cancer cell invasion into the blood vessels and the prognosis: Rarely we discerned cancer cell invasion into the blood vessels (the veins) of the operatively excised uterus, the cases being only 13 out of 278 cases examined. The relation between such blood vessel invasion and the prognosis is as shown in the following table.

	Healthy	Death within 5 yrs.	Total cases
1. stage	1	4	5
2. stage	5	2	7
3. stage	0	1	1
Total	6	7	13

It is worthy of notice that although the cancer development in these cases had gone so for as to invade the blood vessels, 6 out of the above 13 cases were saved by the operation. This certainly speaks for the assumption that a considerable number of cancer cells invading into the blood vessels are destroyed.

An Investigation on the Effect of Biopsy of Caroinoma of Cervix Uteri Upon Cancer Development

Some assert that even such a slight operative invasion as biopsy affects the prognosis, facilitating the cancerous development in some cases. For a greater part of my material, therefore, hysterectomy was performed on what was diagnosed as cancer as soon as possible without any biopsy. Up to the present, however, there are 30 cases, operated upon after the ascertainment of cancer by means of biopsy, and in only 13 of these cases 5 years' clinal course could be traced. On these cases, both biopsy specimen and surgically exstirpated uterus of the same patient are nearly identical on the histologic figures classified by Imai's principle. Out of the 13 cases, which were observed over 5 years after operation, 2 showed hopeless prognosis. In 2 cases only in the surgically exstirpated material was detectable the cancer cell invasion in lymphatic vessels, but with a slight degre—L I type development at the most. This may be explained more adequately from the fact that my examination was performed more extensively in the exstirpated uterus than in the biopsy tissue. Moreover, in the above 2 cases of hopeless prognosis Imai's Schub figure was already discernible in the experimentally excised tissues. It may be a little risky to draw any conclusion from the above few cases, but it may not be going too far to say that biopsy does not facilitate the cancer cell invasion into either blood or lymphatic vessels. At least if hysterectomy is performed immediately after the biopsy the prognosis of uterus cancer is not much affected.

So-Called Parametrial Infiltration.

Carcinoma of cervix uteri is divided clinically into three stages, namely, stages I, II, III according to the degree of the infiltration in the parametrium or the degree of fixation of the uterus as the criteria. It is well known that the so-called infiltration is not entirely cancerous in nature. I made a histological investigation upon the bilateral parametria of 57 cases, namely, 49 cases of epidermoid carcinoma and 8 of adenocarcinoma. Since the adenocarcinoma cases are small in number and also the adenocarcinomas and epidermoid carcinoma showed no variance in this change, they are shown en bloc in the following table. To divide them into the stages of disease, 26 cases belong to stage II and 31 belong to stage III.

As to the method of investigation, the parametrium excised by means of Okabayashi's total hysterectomy modified by Ogino was extended laterally and a frontal section was made as widely as possible along the surface. Since serial sections are not made, some very slight cancerous infiltration may be missed under this method but this may be sufficient for grasping a general tendency.

The chief histologic changes discernible are carcinomatous infiltration, inflammatory cell infiltration, and the proliferation of connective tissue. The following

table shows these changes, the connective tissue proliferation being divided into 3 grades.

Clinical stage	Carcinomatous infiltration	Connective tissue proliferation			inflammatory cell infiltration	Number of research material
		+	++	+++		
Stage II	9 (31.6%)	8	17	1	4 (15.3%)	26
			26 (100%)			
Stage III	13 (41.9%)	2	14	15	11 (35.4%)	31
			31 (100%)			
Total cases	22 (38.5%)	10	31	16	15 (26.3%)	51
			57 (100%)			

The above table shows that out of the total cases of the so-called parametrial infiltration, only 38% are the real carcinomatous infiltration, which is a continuous invasion localized only in the adjacent portion of cervix uteri far from the center of the parametrium, even in the cases of stage III, in which the infiltration is thought to reach the pelvic wall. Thus, the parametrial infiltration is mostly caused by the connective tissue proliferation, only 26.3% being accompanied by inflammatory cell intilatration.

In a very few cases of carcinomatous infiltration, however, the invasion into the perineural lymphatic spaces or the blood vessels was seen. The material here treated with, however, includes no cases with lymph node metastasis.

Then, what is the mechanism for connective tissue proliferation? The chief inflammatory cell infiltration detected here was that of lymphocytes, which mostly show follicular grouping. Although we have to admit that such connective tissue proliferation or lymphocytic infiltration is the trace of some infectious, inflammatory process, here also Imai's anti-cancerous reaction against the invasion of carcinoma of cervix uteri into the parametrial connective tissue seems to be more significant.

Conclusion.

(1) 250 cases of carcinoma of cervix uteri and its metastasis—the material exstirpated by means of Okabayashi's extended hysterectomy modified by Ogino, with the record of clinical course for 5 years' survival after the operation—were brought under a detailed histologic investigation. The parametrial connective tissue thereof and cases of the same kind of cancer were examined histologically and the relationship between these and the prognosis was studied.

(2) I verified stochastically also that the method of determining the malignancy of carcinoma on the basis of its histologic structure, which was suggested and is applied by some American pathologists, such as Broders and Martzloff, has no clinical value.

(3) The number of cancer cell mitoses also has no relation with the prognosis.

(4) The C. P. L. classification of cancerous development suggested by Imai on the basis of his comprehensive study on the cancerous parenchymal development and the stromal reaction against it in the perifocal cancer development is effectively suggestive of the prognostic condition. Especially the prognosis of the cases with marked cell invasion into the lymphatic vessels (so-called L III type) is usually hopeless; similarly that of the cases with marked cancer cell infiltration into the tissue space with no stromal cellular reaction (so-called P III type) is not hopeful; whereas that of the so-called C type, which shows vigorous stromal reaction against the carcinomatous parenchyma is very hopeful.

(5) The carcinoma cells can be generally differentiated from the normal cells of their matrix on the basis of various cytologic findings, but the reliable method of differentiation between the former and the epithelial cells in precancerous stage has not yet been found.

(6) Biopsy of carcinomatous tissue hardly affects the cancerous development.

(7) Consequently, for the diagnosis of uterine carcinoma the simple application of the Papanicolaou method is not sufficient. The final determination should always be based on biopsy

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Explanation of Plates XV and XVI.

Fig. 1. Deeply infiltrated carcerous focus of cervix uteri. Pap's silver impregnation. Around the carcinoma cell nests is seen a typical basement membrane, which is transformed from collagenous fiber.

Fig. 2. Spindle cell cancer developing along the mucosal surface of cervix uteri. Mucous gland is seen remaining under the epithelial layer.

Fig. 3. Primary focus of carcinoma of cervix uteri. Spindle cell carcinoma.

Fig. 4. Metastatic focus of the same case as Fig. 3. Spinal cell cancer.

Fig. 5. Both spindel cell cancer and spinal cell cancer are seen in the same preparation.

Fig. 6. L III type of carcinomatous development. Spinal cell cancer. Cancerous cells are invading in the comparatively large lymphatic vessels. Death 8 months after the hysterectomy.

Fig. 7. P III type of carcinomatous development. Spindle cell cancer. Cancerous cells are infiltrating into the pre-existing tissue spaces and developing there. Death 4 years and 6 months after the hysterectomy.

Fig. 8. L III type development of carcinoma of cervix uteri. Transitional cell cancer. Autopsy material.

Fig. 9. C type development of carcinoma of cervix uteri. Transitional cell cancer. Healthy 8 years and 11 months after the hysterectomy.

Fig. 10. C type development of carcinoma of cervix uteri. Spinal cell cancer. Healty 12 years and 10 months after the hysterectomy.

Fig. 11. L III type development of carcinoma of cervix uteri. Spindle cell cancer. Biopsy material.

Fig. 12. L III type carcinomatous development of the same case as Fig. 11. Spindle cell cancer. Operatively excised material. Death 2 years and 10 months after the hysterectomy.

要　旨

子宮頸癌の病理組織学的諸問題，特に予後との関係

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本研究の主な対象は岡林式広汎子宮剔除術の荻野氏変法を以て荻野自身手術剔出し，術後5年乃至それ以上経過を観察した子宮頸癌278例であるが，他に子宮頸癌屍35例，試験的切除頸癌組織及び子宮頸癌例の膣内容塗抹標本等をも参考に供した。

如上の子宮頸癌手術材料の病期を新国除分類法に従って分けると第I期49例，第II期193例，第III期36例となり，また組織学的分類では腺癌ないし単純癌33例，基底細胞ないし扁平上皮癌245例となる。もっとも組織学的分類については1個の子宮頸癌で全部同一像を示すことはむしろ稀であって，場所により異なる極めて多様な像を示すものが多い。

組織学的分類の基礎をなす各項目を十分吟味した上で，著者の材料につきこの癌の組織学的悪性度に関するBrodersとMartzloffの方法を推計学的に検討した結果，これ等の方法による予後判定には意義を認め難いことを明かにした。また癌細胞の核分割数と予後との間にも何等の相関関係を認めることが出来なかった。

しかるに1949年今井によって提唱された癌発育型のC. P. L.分類と予後との間には極めて密接な関係の存することを推計学的に明かにした。まず癌腫のC型発育型を示したものはその86.5%迄術後5年間生存しているのに対し，L型特にLIII型発育を示すものは同じ期間に94%癌の再発または転移に基き死亡している。なおLI，LII，LIII各発育型の間にも推計学的に明かな有意の差を認めることが出来た。P型発育型についてはPI及びPII型と予後との間には明かな相関関係を見出し難いが，PIII型の発育を示すものでは明白に予後が悪い(手術5年後迄の間に75%死亡)ことを知った。かかる今井のC. P. L.分類の本態についてはなお説明困難であるが，生体の呈する一種の抗癌的作用に帰してよいであろう。すなわちC型発育はかかる作用の強力に存する事を示し，P型及びL型はこれを欠くか，あるいは減弱していることを物語るものと思われる。

次に著者の取扱った手術材料をリンパ節転移の有無によって二群に分け，それぞれ予後との関係を検討して見た結果，リンパ節転の証明された群では60%迄術後5年以内に死亡しているのに対し，転移の認められなかった群では僅かに17%の死亡率を見たに過ぎない。

子宮頸癌細胞の血管内侵入を認めたのは 278 例中僅か 13 例であり，しかもその中の 6 例が 5 年後健存であった。

さらに癌組織の試験的切除が予後に及ぼす影響を切除組織の組織像の C. P. L. 分類と手術的剔除組織のそれとによって比較検討して見た結果，なお例数の過少なうらみはあるが，認むべき影響なしとの結論に達した。

最後に子宮頸癌の臨床的分類の一つの指標とされているいわゆる子宮旁結合織浸潤の本態を 57 例（扁平上皮癌 49 例，腺癌 8 例）について検索して見たのであるが，癌細胞の浸潤を認めたものは第 II 期で 31.6% 第 III 期で 41.9%に過ぎず，特に第 III 期の症例といえども癌性浸潤が骨盤壁にまで到達したものは 1 例もなく，その主体は結合織増殖 (100%) であることを確めた。

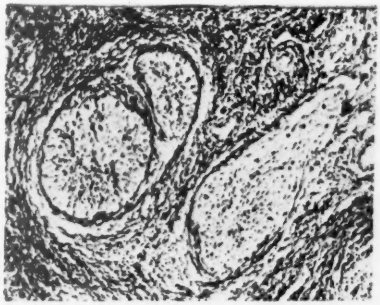

Fig. 1

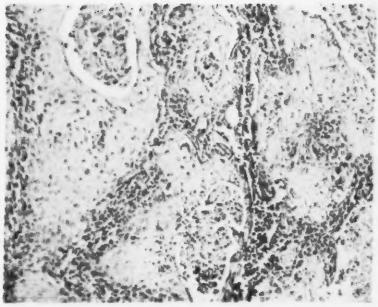

Fig. 4

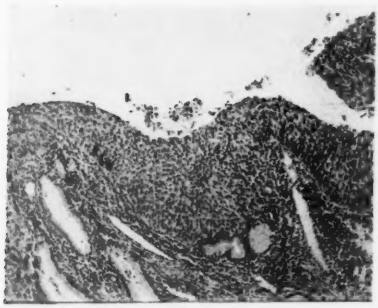

Fig. 2

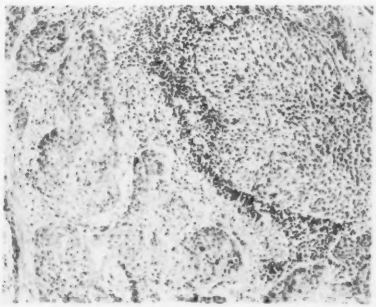

Fig. 5

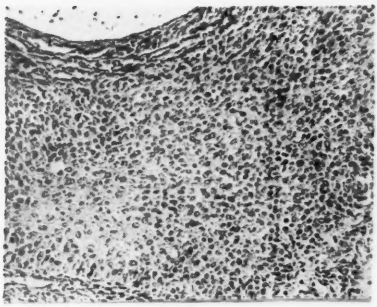

Fig. 3

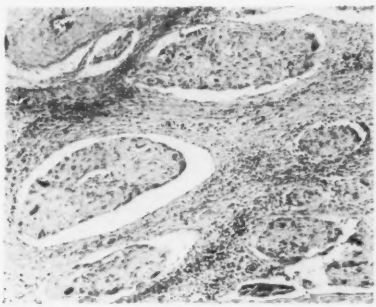

Fig. 6

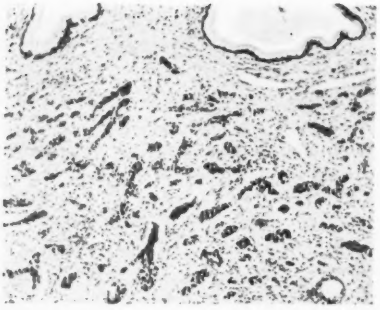

Fig. 7

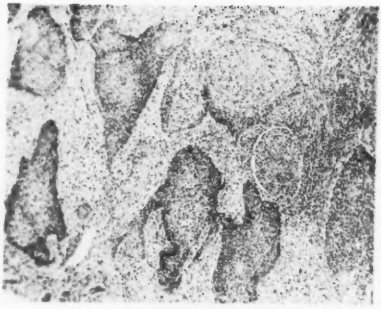

Fig. 10

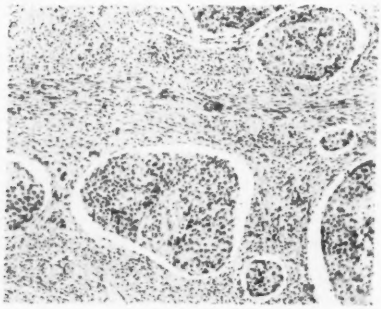

Fig. 8

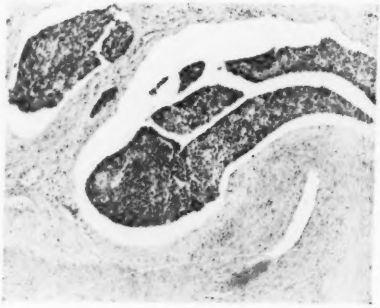

Fig. 11

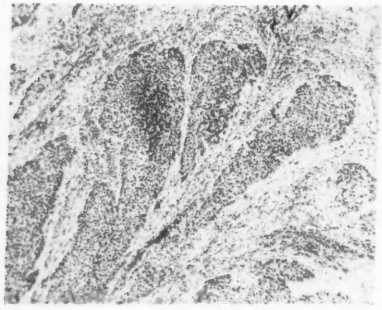

Fig. 9

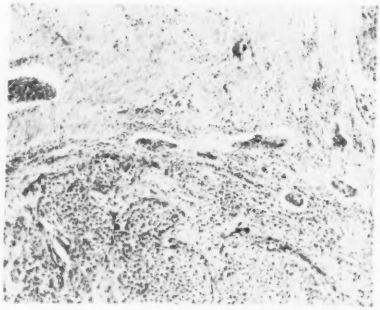

Fig. 12

[GANN, Vol. 44; December 1953]

PRODUCTION OF GASTRIC LESIONS IN THE RAT BY THE DIET CONTAINING FATTY ACIDS*

(With Plates XVII-XXII)

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The development of gastric lesions in rats receiving diets containing fatty acids, such as propionic, butyric and valeric acids, was demonstrated in this laboratory. The results are reported in this paper.

Experimental Procedures

The fatty acids fed to rats were propionic, butyric, valeric, capronic, capric, lauric and palmitic acids. And the severe hyperplasia of the forestomach of rats developed only in the rats which were maintained on the diet containing low fatty acids, namely, propionic, butyric and valeric acids, while none of the rats receiving other fatty acids listed above showed such a gastric lesion.

Both sexes of albino rats of mixed strain weighing 50 g to 80 g were maintained on rice diet, to which the fatty acid was mixed evenly. Usually, the amount of the fatty acid added was 5–10 per cent of the rice. But, in the case of the butyric acid, the initial amount was 1 per cent of rice, and it was gradually raised up to 10 per cent by two week intervals after the beginning of the experiment. A small amount of cod liver oil was introduced along with the fatty acid on the assumption that the dissipation of fatty acid from the surface of rice grains may be somewhat hindered by so doing and that the diet may not become deficient in vitamin A. No attempt was made to estimate the actual amount of fatty acid ingested by the rats as the volatility of fatty acid rendered any such attempt practically impossible. In view of the deficient nature of the diet, green vegetables were given to the rats from time to time.

The toxicity of fatty acids is not great when given with food and essentially when the amount is five per cent or less animals seem to thrive and show substantial increase in body weight. The body weight of animals was measured every two weeks.

In the course of the experiment the rats were killed by exsanguination. The stomachs were removed at once and placed in a formalin fixative. After slight

* Aided by a Scientific Research Encouragement Grant from the Department of Education.

fixation, the stomachs were cut along the greater curvature, and examined for gross lesions. Then, those showing gross abnormalities were taken for routine histological examination, using hematoxylin-eosin stain.

Results

1. Butyric acid diet.

In this experiment, the amount of butyric acid added to rice varied. It was started with a small amount, 10 cc per 1 kg of rice, and was gradually raised up to 100 cc per 1 kg. Duration of each dose was about two weeks. In one group of rats which were killed on the 350 th day, the diet was supplemented with the powder of the dried beef liver, brewer's yeast and cod liver oil to avoid the deficient nurishment.

One hundred and twenty rats receiving the butyric acid diet were killed on the various experimental periods: 10 rats on the 50 th day, 5 on the 75 th day, 35 on the 100 th day, 15 on the 135 th day, 18 on the 150 th day, 10 on the 350 th day and 5 on the 500 th day, and several examples of these rats are shown in the plates. Twenty-two rats died on the day between these experimental periods. The stomachs of these experimental rats were examined for gross lesions and were fixed in formalin, were embedded in paraffin and cut and stained routinely. Figs. 1–10 show the lesions in the forestomach of rats kept on butyric acid diet for various durations.

The gastric lesions of the rats which were killed on the various periods of experiment are summerized in Table 1. As shown in the table, the majority of the rats which received butyric acid diet more than 50 days showed more or less definite gastric lesions, such as hyperkeratosis, hyperplasia and papillomatosis. And the lesions of papillomas with keratin cysts are often developed in the rats of

Table 1. Gastric Lesions in the Forestomachs of Rats kept on Butyric Acid Diet.

Experimental Days	Number of Rats	Gastric Lesions in the Forestomach		
		Hyperkeratosis or Hyperplasia only	Moderate degree of papillomatous or warty lesions	Extensive papillomatous growths with numerous keratin cysts
50	10	1	8	1
75	5	0	5	0
100	35	3	30	2
135	15	0	6	9
150	18	3	13	2
350*	10	0	1	9
500	5	0	0	5

* Butyric acid diet was supplemented with beef liver powder, brewer's yeast and cod liver oil.

more than 135 experimental days.

In the rats fed butyric acid diet for a long period, 350 and 500 days, marked keratin cysts were easily visible on the serosal view. In the case of rats which were killed on the 350th day, the degree of the gastric lesions was similar to that of 500 day case. Then it seemed that sufficient diets of beef liver, brewer's yeast and cod liver oil did not favour the occurrence of the lesions but the responsible factors could not be analysed. These gastric lesions, however, did not change into malignant even when the administration was long in period and the condition was very pronounced. No remarkable change was found in the glandular stomach.

2. Propionic acid diet.

In this experiment, only 5 rats were maintained on rice, to which propionic acid was added. The amount of propionic acid added was 50 cc per 1 kg of rice throughout the experiment. One rat died early in the course of the experiment, and remaining 4 rats were killed on the 110th day. The result is shown in Table 2. In 3 of 4 cases, umbilicate or warty lesions were evident in the forestomach, and the remaining one, however, showed no gastric change. These lesions to the naked eye appeared as isolated crater-like warts with ulceration on their tops. Figs. 11–13 show these lesions.

Table 2. Gastric Lesions in the Forestomachs of Rats kept on Propionic and Valeric Acid Diets.

Diet	Experimental Days	Number of Rats	Gastric Lesions in the Forestomach		
			Macroscopically Normal	Umbilicate or warty lesions	Extensive papillomatous growths
Propionic Acid	110	4	1	3	0
Valeric Acid	115	5	2	0	3
	150	4	0	0	4

3. Valeric acid diet.

10 rats were administered on rice, to which valeric acid was added. The amount of valeric acid added was 50 cc per 1 kg of rice throughout the experiment. One rat died early in the experiment. Five rats were killed on the 115th day, and the remaining four were sacrificed on the 150th day.

The results of the above experiment are shown in Table 2. In the table, 3 of 5 rats which were 115 day cases showed umbilicate lesions scattered in the forestomachs and 2 others revealed no lesion. All 4 rats of 150 day period, showed remarkable papillomatous growths. These lesions caused by valeric acid feeding, however, were not accompanied with keratin cyst which was usually seen in the

butyric acid case. Figs. 14–16 show these cases.

4. Other fatty acids diets.

Ten rats each were administered the rice diet, to which capronic, capric, lauric, or palmitic acids was added each in the proportion of 10 per cent. In all cases, the duration of the administration was 150 days. No remarkable change was detected either in the forestomach or glandular stomachs of rats kept on these fatty acid diets.

Pathological Notes

Grossly, many of the forestomachs were slightly enlarged, the wall were opaque, gray or white, and indurated, showing numerous irregular protuberances on the external surface. When the stomachs were cut along the greater curvature, hyperplasia of the epithelial lining and large papillomatous growth were grossly evident occupying a large portion of forestomach of all rats. In the early course of the experiment the discrete umbilicate lesions were seen. These lesions varied from small to large sizes. To the naked eye these lesions appear as numerous papillomata or warty excrescences. And the limiting ridge was prominent. The glandular stomach, however, was quite intact in all experimental groups.

Microscopically the squamous epithelium lining of the forestomach showed hyperkeratosis or hyperplasia which were designated as the diffuse lesions The retia were increased numerically, elongated and broadened, sometimes interlaced and anastomosing, giving the appearance of sessile papillary formation. The characteristic lesion was papillomatosis which were designated as the umbilicate or warty lesion frequently found in the forestomachs. Occasionally, spurs of hyperplastic epithelium extended downward from the papillomatous growths and penetrated the muscularis mucosa (Fig. 9). Some of these were found deeply placed in the submucosa heterotopically. When rats were administered with butyric acid for more than 135 days, these proliferations were covered with keratin in concentric whorls and in a flamelike fibrillary arrangement (Fig 10). These keratin cysts, large or small, were found penetrating the muscularis mucosa. Very often, these cysts were grossly visible on the outside surface of the stomach. And, it is noteworthy that the keratin cyst was prominent in the case of butyric acid only.

Generally speaking, these gastric lesions which developed in rats fed low fatty acids are similar in kind. But it is most severe in the butyric acid feeding. And the lesions in the case of propionic acid feeding seemed to be rather destructive than those due to butyric or valeric acid. And the valeric case is less marked than butyric case in the grade. In all foregoing gastric lesions, no evidence of true infiltrative malignant growth was detected in any part.

Discussion and Conclusions

Since the discovery of Fibiger, many workers have tried by various means to induce malignant tumors in the stomachs of rodents, but for the most part without succes. And reviews of the literature have been published by Klein and Palmer,[1] Sugiura,[2] Ivy,[3] Barrett,[4] and recently by Peacock et al.[5] Salmon and Copeland[6,7] appeared to be the first to have described that the marked gastric lesions were induced in the forestomach of rats which were kept on diet of large amount of tributyrin or butyic acid supplement. The author[8] indicated previously that the slight lesions in the forestomach of rats were produced when acetic acid was added to the diet. In this paper the effect of the administration of various fatty acids to rats was investigated. And the development of the lesions in the forestomach of rats was demonstrated in the case of administration of low fatty acids, i. e., propionic, butyric and valeric acids. And these results in the butyric acid are in accordance with reports of Salmon and Copeland. These lesions however are not entirely due to the vitamin A deficient condition.[9]

Microscopic examination of the gastric lesions revealed mainly extensive benign hyperplasia, hyperkeratosis of the squamous epithelium lining, consisting in the development of numerous papillomata and warty excrescences and occupying a large portion of the forestomach. These lesions are most severe in the case of butyric acid among the fatty acids investigated for comparable periods of time. The gastric lesions caused by propionic acid feeding was somewhat destructive in nature. And valeric acid produced less damage to the epithelial lining than butyric acid. Numerous keratin cysts were found only in the case of butyric acid diet. However, the glandular stomach of rats receiving these fatty acids remained quite intact notwithstanding the lining of forestomach was extensively damaged. No remarkable change was found as the result of administration of high fatty acids, such as capronic, capric, lauric and palmitic acids.

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Explanation of Plates XVII and XXII.

Fig. 1. Gross mucosal appearance of stomachs of rats kept on butyric acid diet for 50 days. Note the several small crater-like warts in the forestomachs.

Fig. 2. Gross mucosal appearance of stomachs of rats kept on butyric acid diet for 100 days. Note the numerous irregular protuberances on the forestomachs.

Figs. 3 and 4. The serosal and mucosal views of stomachs of rats kept on butyric acid diet for 350 days. Outside of the stomachs, several keratin cysts are visible, and the entire forestomachs are masses of extensive papillomas.

Fig. 5. The mucosal aspect of stomach of rat kept on butyric acid diet for 400 days. Note the keratin cysts and papillomas.

Figs. 6 and 7. The serosal and mucosal views of stomachs of rats kept on butyric acid diet for 500 days. One of them showed ulceration.

Fig. 8. Section through papilloma covered with hyperkeratotic lining of the epithelium. (Butyric acid 100 days).

Fig. 9. Section through papilloma showing a massive proliferation of straitified squamous epithelium, hyperkeratosis, and heterotopic growth. (Butyric acid 500 days)

Fig. 10. Section through advanced papilloma showing cystic growths lined with straitified squamous epithelium and containing keratins arranged in fibrillary lamellae. (Butyric acid 500 days).

Fig. 11. Two examples of stomachs of rats kept on propionic acid diet for 110 days. Note the crater-like warts in the forestomachs.

Fig. 12. Section through crater wart, showing inflammatory focus at the keratin epithelial junction, a break in the keratin above, and hyperemia and inflammatory cells in the lamina propria. (Propionic acid 110 days).

Fig. 13. The diffuse lesions is characterized by hyperkeratosis and elongation of retia. Note the heterotopic growth of the squamous epithelial cells. (Propionic acid 110 days).

Fig. 14. The mucosal aspect of stomachs of rats kept on valeric acid diet for 115 days. Note the crater-like papillomatous growths.

Fig. 15. The mucosal aspect of stomachs of rats kept on valeric acid diet for 150 days. Note the extensive papillomatosis.

Fig. 16. Section through papilloma to show proliferation of stratified squamous epithelium. (Valeric acid 150 days).

要　旨

脂肪酸添加飼料による白鼠の前胃変化

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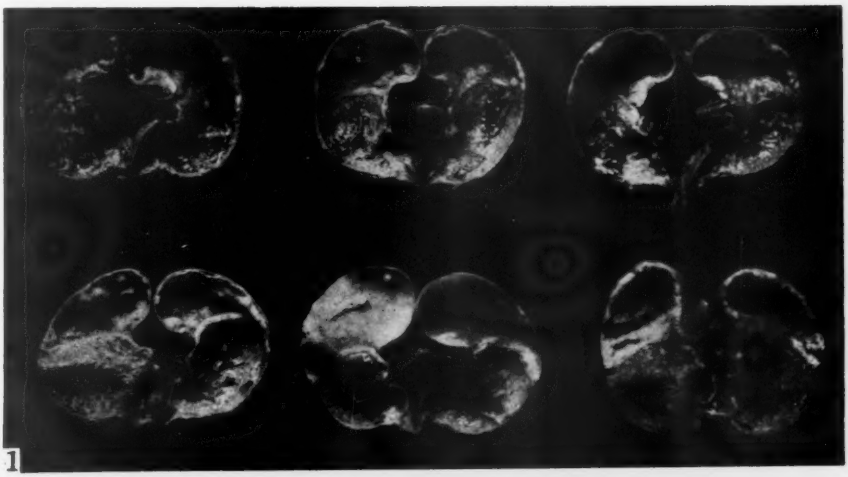
(昭和医科大学　医動物学教室)

白米に5~10%の割合で脂肪酸を添加した飼料を白鼠に与えることによって，動物の前胃粘膜に乳嘴腫様変化が生成されることが確められた。実験に際し，脂肪酸としては，プロピオン酸，酪酸，吉草酸，カプロン酸，カプリン酸，ラウリン酸並びにパルミチン酸を選んだ。しかし，実験の結果，いわゆる低級脂肪酸（プロピオン酸，酪酸並びに吉草酸）飼与の場合にのみ動物の前胃に変化がみとめられ，他の高級脂肪酸の際には著変がみられなかった。

これらの前胃変化は，いづれの脂肪酸の場合でも大体同じ傾向を示し，扁平上皮細胞の著しい角化並びに肥厚増殖が先行し，さらに乳嘴腫様変化にまで発展する。酪酸飼与の場合に限り，特徴ある Keratin cyst の出現がみられる。しかし 500 日に亘る長期の飼養にも拘らず，腫瘍化することはなかった。



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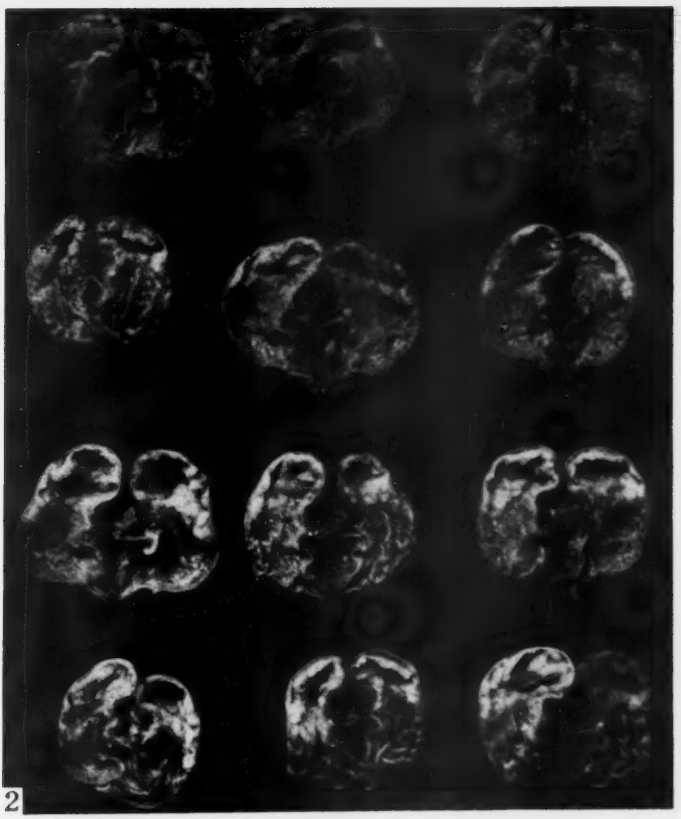

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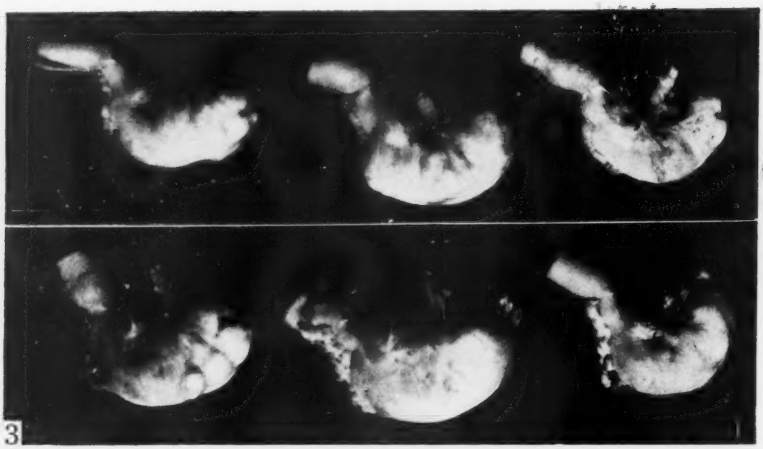

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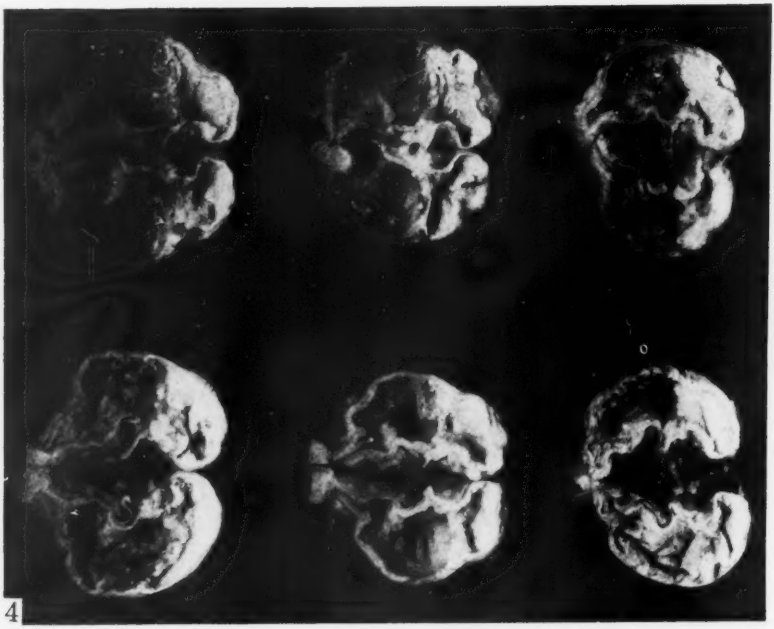

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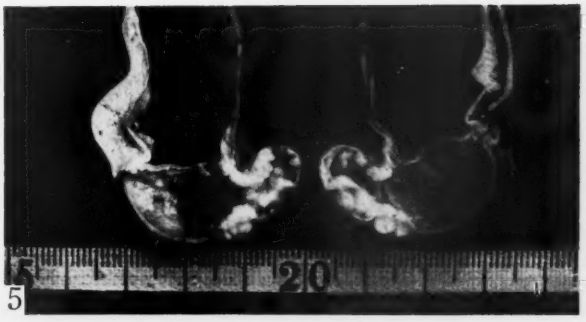
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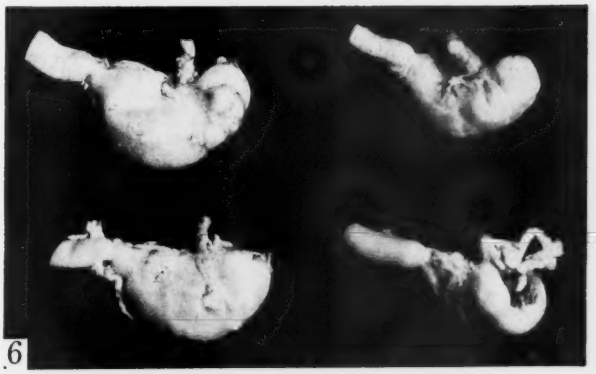
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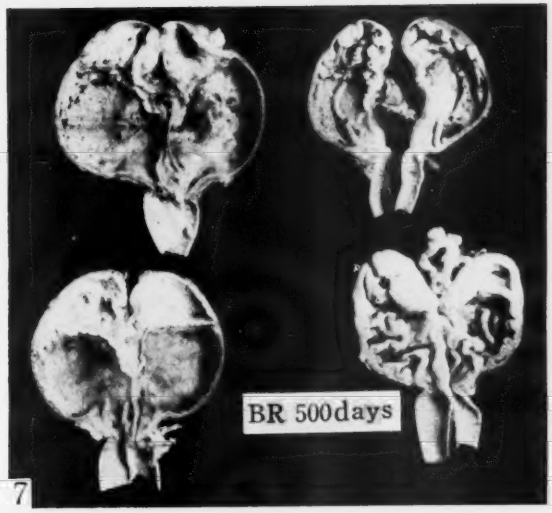
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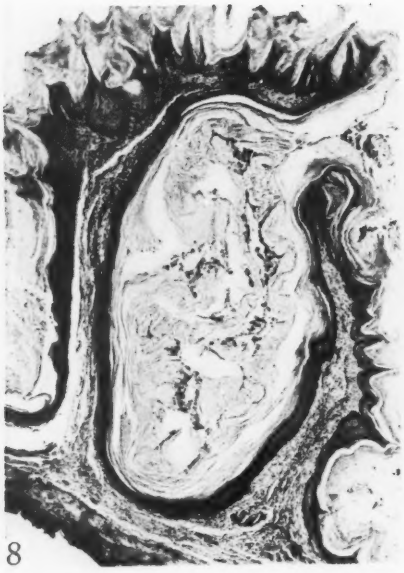

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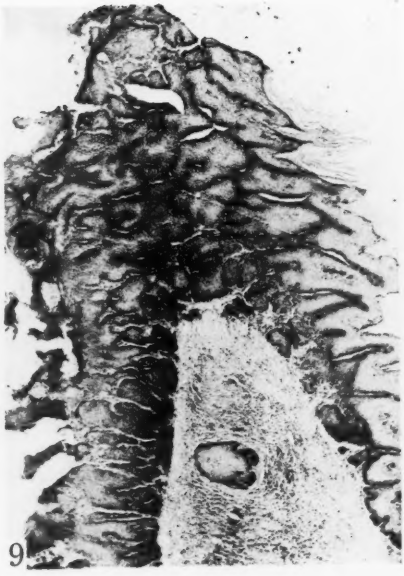

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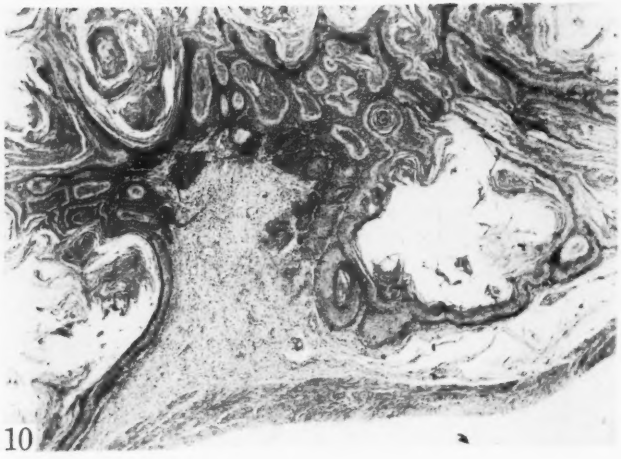

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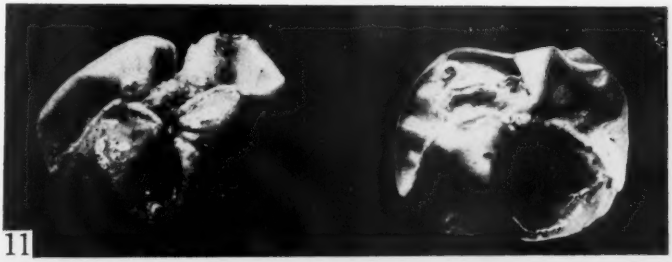

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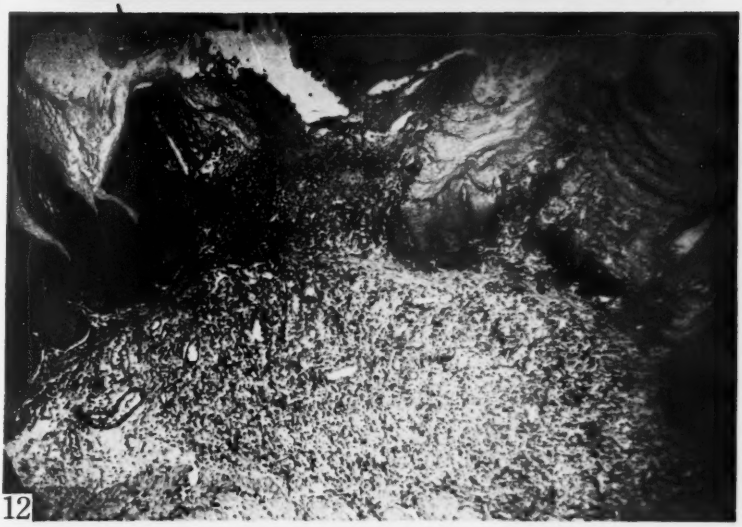

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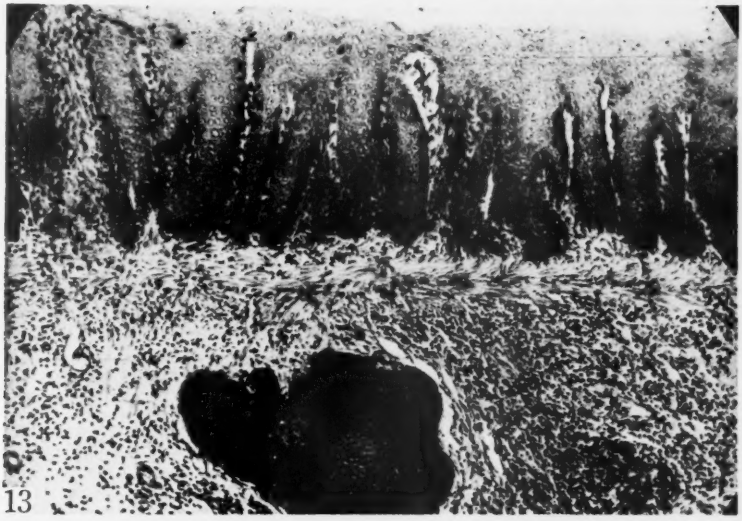

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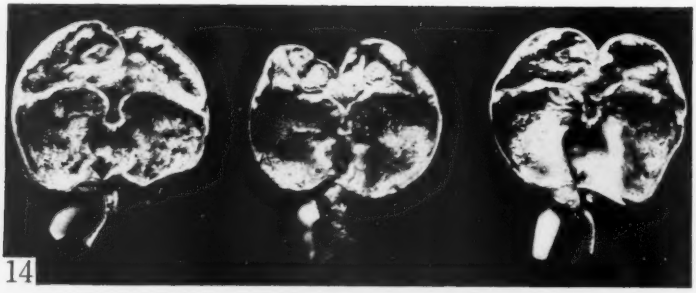

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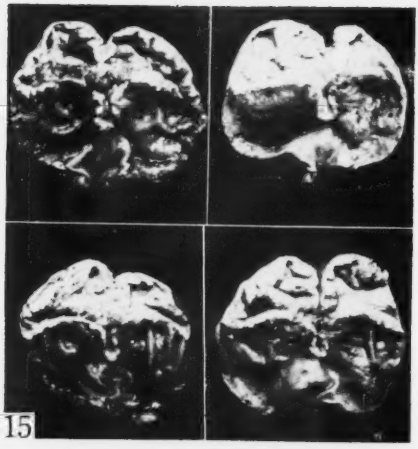

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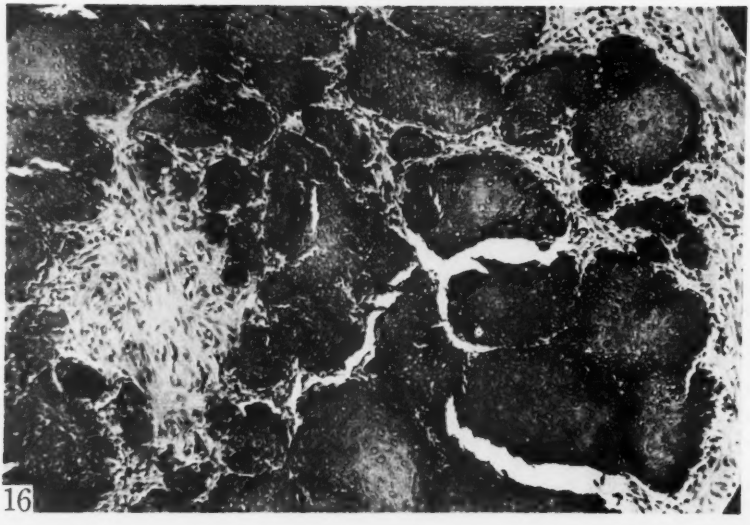

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[GANN, Vol. 44; December, 1953]

INHIBITION OF EXPERIMENTAL PRODUCTION OF LIVER CANCER BY ADDITION OF ACETIC ACID TO THE DIET*

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Since the discovery of the production of the liver cancer using certain azo-compounds by Sasaki and Yoshida,[1] and Kinoshita,[2] numerous attempts to inhibit the liver cancer resulting from the feeding of these dyes have been demonstrated by many investigators. And their results have indicated the possible existence of liver cancer inhibiting substances in vegetable and animal materials.[3-14] Several of these expriments show that the diet may have some effect on the experimental liver cancer production, but, so far as the author is aware, no such drastic inhibition as effected by feeding liver[3,5,11,15] and kidney[8] has ever been brought about by any other dietary means. The inhibiting effect by feeding liver was to such an extent as to prevent even the development of cirrhosis and to keep liver macroscopically normal for 150 days and more. The present experiment has demonstrated that the experimental production of liver cancer is inhibited by the diet supplemented with acetic acid. The details of this experiment are given in the following pages.

EXPERIMENTS

Three groups of male and female albino rats ranging from 60 to 80 g in weight were used for this experiment.

The first group: 60 rats were maintained on unpolished rice (1 kg) evenly mixed with p-dimethylaminoazobenzene (butter yellow) (0.6 g) dissolved in glacial acetic acid (50 cc).

The second group: 10 rats were kept on the acetic acid butter yellow diet, but in this group acetic acid was added at the level of 2.5 per cent. In these groups (groups 1 and 2), when the p-dimethylaminoazobenzene was dissolved in glacial acetic acid, the color of the solution turned into crimson red. But this red color again turned back to yellow within five minutes when the butter yellow-acetic acid solution was mixed with rice.

The third group (control): 40 rats were fed on the mixture of unpolished rice (1 kg) and methanol (50 cc) in which p-dimethylaminoazobenzene (0.6 g) was dissolved.

In view of the deficient nature of the diet, a small amount of the cod liver oil was added to each. And all experimental animals were given green vegetables

* Aided by a Scientific Research Encouragement grant from the Department of Education.

thrice a week and water ad libitum. Some of the rats died early in the course of the experiment, too early to show any relevant change, and they were discarded. Among the first group, 10 on the 120th day, 15 on the 140th day and 25 rats on the 165th day were killed by carotid bleeding and autopsied. The second and third groups were terminated on the 150th day after the commencement of experiments by sacrificing all the rats in order to make a final comparison of liver changes between these groups.

The results of the above experiments are summarized in Table 1, where the nature of liver findings is tabulated in the following four degrees: liver cancer, cirrhotic change only, slightly uneven surface without extensive proliferation of connective tissue, and macroscopically normal. The identification of these liver findings has been described in detail in the publication cited.[5]

In the first group, 9 (90.0%) among the total 10 rats which were sacrificed on the 100th experimental day showed apparently normal liver and in the remaining 1 case (10.0%) the liver change was not advanced beyond the stage of somewhat granular and uneven surface. 8 (53.5%) among the total 15 rats which were killed on the 140th day showed macroscopically normal livers, 4 rats (26.6%) showed slightly granular livers, and in the remaining 3 cases, the localized or annular cirrhosis of the liver was seen. Among the total 25 rats which were killed on the 165th day, 21 (84.0%) showed macroscopically normal livers, and the liver surface in the other 3 cases (12.0%) was slightly granular, and only in the remaining 1 case (4.0%) cirrhotic change was advanced. No liver cancer was produced in the rat of this group.

A somewhat notable feature of the experimental results was that the slight splenic enlargement was indicated in the rats fed on acetic acid diet at the level of 5 per cent, notwithstanding the absence of notable change in the liver size as shown in Table 2. The average of the ratio of the spleen to the total body weight of the experimental animals was 1.07, more than twice that of the normal (0.3–0.43).

Against the results of the first group, no inhibition of the production of liver cancer was demonstrated in the second group in which the dye diet was mixed with the acetic acid at the level of 2.5 per cent. As is shown in Table 1, the second group included 4 cases (40.0%) of liver cancer accompaning cirrhosis, 4 cases (40.0%) of typical cirrhosis and the remaining 2 cases were beyond the stage of somewhat granular and uneven surface.

Then, the findings in the third group (control) were as follows: 9 cases (29.0%) of macroscopically normal, 5 cases (16.3%) of uneven surface, 7 cases (22.6%) of typical cirrhotic and 10 (32.3%) of liver cancer accompaning annular cirrhosis.

The average amount of food intake per rat per day in the first group (acetic acid group) was 7.7 g and that of the third (control) was 8.9 g. And it is thought that the difference within this range in the amount of food intake will not affect

Table 1. Comparison of Liver Changes between Acetic Acid Fed and Control Dye-diet Groups.

Experimental Group	Experimental Days	No. of rats	Liver Findings			
			Macroscopically Normal	Liver with Slightly uneven surface	Cirrhotic Liver	Liver Cancer
First Group Dye-diet with 5% acetic acid	100	10	9 (90.0%)	1 (10.0%)	0	0
	140	15	8 (53.5%)	4 (26.6%)	3 (20.0%)	0
	165	25*	21 (84.0%)	3 (12.0%)	1 (4.0%)	0
Total		50	38 (76.0%)	8 (16.0%)	4 (8.0%)	0
Second Group Dye-diet with 2.5% acetic acid	150	10	0	2 (20.0%)	4 (40.0%)	4 (40.0%)
Third Group Dye-diet only (Control)	150	31	9 (29.0%)	5 (16.3%)	7 (22.6%)	10 (32.3%)

* The weights of the body, liver and spleen of these rats are show in Table 2.

the degree of the liver cancer production, according our experience of these ten years.

The results of these experiments described above clearly demonstrated that the acetic acid at the level of 5 per cent in the dye diet has inhibited markedly the production of liver cancer, but when the amount of acetic acid was reduced to 2.5 per cent in the diet, the inhibiting effect failed completely.

The above result leaves little doubt as to the marked inhibiting effect which acetic acid feeding at the level of 5 per cent exerts on the production of liver cancer by oral administration of p-dimethylaminoazobenzene. In this experiment, a question arose as to the possibility of butter yellow being destroyed while it is kept in contact with acetic acid in the diet mixture.

As described above, the glacial acetic acid, in which butter yellow was dissolved, mixed with rice and the rats were allowed to feed upon the mixture ad libitum. It is not entirely without reason to suspect that this carcinogen might suffer some chemical modification by the contact with glacial acid outside of the animal body, with the resultant loss of the carcinogenecity before it enters into the body of the animal. Here is the experimental datum, demonstrating that butter yellow is by no means destroyed by being kept mixed with acetic acid and rice. Out of 100 g of the acetic acid-butter yellow rice, 65 mg of butter yellow was indicated (theoretically, a 100 g portion of the original mixutre contains 60 mg of butter yellow), and the crystals isolated had M. P. 115°C, agreeing with that of pure p-dimethylaminoazobenzene. The method of recovery of the azo dye used here was the same

Table 2. Body, Liver and Spleen Weights and their Ratios in the Rats fed Acetic Acid Dye Diet killed on the 165th day.

Rat No.	(A) Body weight (g)	(B) Liver weight (g)	(C) Spleen weight (g)	Liver ratio B/A x100	Spleen ratio C/A x100	Liver Finding
1	95	5.5	1.0	5.8	1.5	Macroscopically normal
2	110	6.8	1.7	6.2	1.5	〃
3	130	8.3	1.5	6.4	1.1	〃
4	140	6.9	1.6	4.6	1.1	〃
5	145	8.5	2.2	5.8	1.5	〃
6	155	7.8	1.4	5.0	0.9	〃
7	160	7.1	1.7	4.4	0.1	〃
8	165	7.9	2.1	4.8	1.3	〃
9	170	9.0	1.9	5.3	1.1	〃
10	170	9.2	2.3	5.4	1.3	〃
11	175	9.0	1.7	5.1	1.0	〃
12	180	7.7	2.0	4.3	1.1	〃
13	180	10.0	1.5	5.6	0.8	〃
14	185	9.5	0.8	5.1	0.5	〃
15	190	8.5	1.0	4.5	0.5	〃
16	190	10.5	1.7	5.5	0.9	〃
17	200	11.4	2.5	5.7	1.2	〃
18	210	10.1	1.5	4.8	0.7	〃
19	215	10.8	1.8	5.0	0.8	〃
20	220	14.0	2.2	6.3	1.0	〃
21	235	10.6	2.0	4.5	0.8	〃
22	95	7.8	0.9	8.2	0.9	uneven surface
23	130	9.4	1.9	7.2	1.4	〃
24	175	10.2	1.5	5.8	0.9	〃
25	120	9.7	1.8	8.0	1.5	Liver cirrhotic

as to that of Nakahara and Kishi.[15]

Nakahara and Fukuoka,[16] and Miyaji and Nakajima[17] have demonstrated that the marked increase in the catalase activity of the liver of rats fed certain diet, for instance the diet containing the beef liver powder, will reduce the production of liver cancer. The average of catalase activity of ten rats fed the acetic acid diet (5 per cent) for the period of 30 days was 6.8 cc in oxygen unit, while it was 5.8 cc in rats of normal diet.[18]

Recently, the development of a severe hyperplasia of the forestomach in rats receiving diets containing acetic acid only was observed in this laboratory.[19] But these gastric lesions were less remarked or not produced in the rats which were fed acetic acid-butter yellow diet (group 1) than the rats fed acetic acid only.

Discussion and Conclusions

Experimental studies on the dietary influence on the genesis of cancer have so far yielded a few significant leads,[13] and yet they remain a problem one of the most important in the field of cancer research. In the previous papers,[3,5,8] it was demonstrated that the liver and kidney feeding brought about a definite inhibition of liver cancer production by p-dimethylaminoazobenzene. In the present investigation dealing with liver cancer production by p-dimethylaninoazobenzene, it has been demonstrated that the acetic acid feeding inhibited the process of liver cancer production, at the level of 5 per cent concentration in the diet. And it is thought that this result was not due entirely to lowered intake of the carcinogen. However no inhibition of liver cancer production took place with the concentration of the acetic acid was reduced to 2.5 per cent.

It is of special interest to note that the acetic acid is the simplest chemical among the dietary supplements hitherto used, such as, corn oil[20,21,22], olive oil[22], lauric acid[23,24], choline[25], riboflavin[26,27,28], thiosalicylic acid, thiouracil[27] and others[29], by which the marked inhibition or retardation of the experimental liver cancer production was brought about.

How the acetic acid feeding brings about the inhibition of cirrhosis or hepatoma production is indeed a complex problem, and the investigation is now under way in this laboratory.

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要　旨

醋酸添加による実験的肝癌生成の抑制

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Azo 化合物に肝癌成生実験に際し，その基本飼料としての穀類を種々かえるとか，あるいは白米飼料にいろいろの物質を添加することによって，肝癌の生成を抑制する報告が従来多くなされている。本研究では，飼料に一定量の醋酸を加えることによって，かなり著しい肝癌生成抑制の効果を認めたことを報告する。

白鼠に p-dimethylaminoazobenzene (Butter yellow) を混じた白米飼料を与え，肝癌生成実験を行った。この際 Butter yellow 0.6 g を氷醋酸 50 cc にとかし白米 1 kg に混じた飼料を与えた群 (第1群)，同量の Butter yellow を 25 cc の氷醋酸にとかし白米 1 kg に混じた飼料を与えた群 (第2群)，並びに対照としてメタノール 50 cc に Butter yellow 0.6 g をとかし白米 1 kg に混じた群 (第3群) の3群にわけ，それぞれ50匹，10 匹並びに30匹の白鼠を飼養した。各群共少量の肝油を与えたこと，週3回の野菜と常に水を与えたことは同様であった。実験の早期に死んだ動物は別として，第1群の動物のうち 10 匹は 120 日目に，8 匹は 140 日目に，さらに 25 匹は 165 日目に出血死せしめ剖見した。第2群並びに第3群は 150 日目に実験を打切り動物の肝所見を第1群のそれと比較した。

肝所見は肝癌，肝硬変，表面不平滑肝並びに肉眼的正常肝の4段階に大別し，実験の結果は第1表に総括した。表に明かなように Butter yellow 白米食に 5% の割合に醋酸を添加することによってかなりの程度の発癌抑制があることが証明された。これらの実験結果から判断して飼料に醋酸を混じた際，Butter yellow が動物に摂取される前にすでに分解されるのではないかという疑問があったため，Butter yellow 醋酸飼料から Butter yellow の回収を試みたところ，量的並びに質的に全く一致した結果を得たので上記の懸念は一応解消した。

Butter yellow 発癌抑制に関し，Catalase の意義が相当重大であるので醋酸 5% を含む白米飼料を 30 日間与えた白鼠の肝 Catalase を測定したところ，正常肝より多少うわまわる値を示した。

要するに 5% 程度の醋酸が飼料に添加されると Butter yellow 肝癌の生成が極度に抑制される。このことは従来抑制物質として知られているもののうち，その構造が最も簡単である点で注目に値すると思う。

[GANN, Vol. 44; December, 1953]

EFFECT OF POLYSACCHARIDE ON THE YOSHIDA SARCOMA CELLS (Preliminary Report)

(With Plates XXIII and XXIV)

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In an extensive study on the type-specific polysaccharide of the Capsular Pneumococcus, Heidelberger, Kendall, Avery, Mc Leod and others had elucidated the fact that the bacterial polysaccharide served as the hapten immunologically, and studied further the possibility of applying this knowledge to clinical therapy. Recently, the study of polysaccharide is being made from various angles. Matsubara et al contended, as the result of their serial studies on fractional components of the neoplastic tissue, that their method was helpful in the early diagnosis of cancer and pregnancy. Recent success of E. J. Hehre in synthesizing polysaccharides promotes the further expansion of our knowledge in this field. While undertaking a study on the specific activity of the bacterial polysaccharide on single cell, the authors examined the specimens of polysaccharide prepared from sarcoma tissue for their effect on the growth of the sarcoma. This study has been motivated by an expectation that the tumor-inhibitory effect of such specimens, if there may be any, might be tumor-specific, and there may be less side-actions in this case than in the case of using other agents.

As the source of polysaccharide, Yoshida sarcoma (ascites sarcoma of the rats) was used.

EXPERIMENTAL

1. Preparation of polysaccharide

Yoshida sarcoma, as growing subcutaneously, was pooled, minced and homogenized. The homogenate was added to 5 volumes of distilled water, and was allowed to stand overnight at 0° C. The supernatant liquid after centrifugation was removed, the precipitate was washed with water repeatedly, and the washings were added to the supernate. Every 100 cc of the combined liquid was mixed with 10 g $CH_3COONa \cdot 3H_2O$ and 1 cc CH_3COOH, and shaken vigorously after addition of 1 to 1.5 volume of 95% ethyl alcohol. After standing overnight in the refrigerator, the supernatant liquid was removed by means of siphon, and the residue was centrifuged. The precipitate after centrifugation was dissolved in 50 cc distilled water, 5 g $CH_3COONa \cdot 3H_2O$ and 0.5 cc CH_3COOH added, and shaken with a mixture of 10 cc $CHCl_3$ and 1.6 cc

buthyl alcohol (normal) $CH_3 \cdot (CH_2)_2 CH_2 OH$ for 1 to 2 hours. Centrifugation at 2000 r.p.m. for 30 minutes to 1 hour resulted in the separation of the liquid into chloroform and n-buthyl alcohol and shaken. This procedure was repeated until the aqueous fraction became negative to Biuret reaction, whereupon it was precipitated by 95% ethyl alcohol. The chloroform fraction was pooled, centrifuged repeatedly to eliminate the aqueous component. The above precipitate dissolved in 20 cc distilled water, was added to 1 to 1.5 volume of alcohol. White mass of precipitate forming in this mixture was removed by centrifugation at 2000 r. p. m. for 15 minutes. When the supernate was mixed with 4 g $CH_3COONa \cdot 3H_2O$ and 0.4 cc CH_3COOH, polysaccharide formed.

Specimens of polysaccharide were examined then for the presence of starch or glycogen by iodine reaction, and for phosphate radicals by ammonium molybdate test. When these tests were positive, specimens were dissolved in distilled water and precipitation was repeated with acetic acid. When negative, addition of redistilled alcohol gave a pure precipitate of polysaccharide. The precipitate was washed again with alcohol, and dried in vacuum with $CaCl_2$ and then with P_2O_5.

Polysaccharide was obtained as white powder, water soluble and alcohol-insoluble. Yield :

Sarcoma	Polysaccharide obtained
32 g	23 mg
45 g	116 mg
36 g	90 mg
22 g	65 mg

2. Animal Experiment

Four to five days after rats were given the intraperitoneal inoculation of Yoshida sarcoma, they were injected with polysaccharide intraperitoneally in dose of either 15 mg, 40 mg or 60 mg in the form of saline solution. Specimens of peritoneal fluid were withdrawn 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, 24 hours and 36 hours after the injection, and were examined for the changes of cells, whereby the tumor-inhibitory activity of polysaccharide was compared with that of nitrogen mustard.

3. Effect of Polysaccharide

Administration of polysaccharide was nearly innoxious to animals, as judged from the result of the intraperitoneal injection, and the manifestation of the direct effect on tumor cells took place only slowly. However, when a fairly large dose was administered, the cytoplasm of cells, both in mitosis and at rest, were subject to specific alteration. Namely, immediately following the leukocytic response 3 to 6 hours after the injection, the maximal inhibition occurred. The number of tumor cells decreased gradually thereafter, becoming sparse after 12 hours. However,

the inhibitory effect expired at the latest 14 to 15 hours later, and gave way to the resumption of tumor cell proliferation, in case no repeated injections were instituted.

The cytoplasm was the only part involved in the pronounced morphological changes such as disappearance, plasmolysis, edema, swelling and deformity, present occasionally associated with maximal inhibitory activities. These findings are highly suggestive of a specific reaction occurring between polysaccharide and the organic constituents of the cytoplasm of tumor cells, although there is no information as to what the underlying mechanism will be. That a minority of tumor cells remained free from any alteration, and that the resumption of tumor cell proliferation took place at the time when inhibitory activity expired, suggest the fact that polysaccharide is of no effect on a certain phase of the cell development (probably the most active, young cells or such cells as holding a particular constitution of the ingredients where the cells are not susceptible to the action of polysaccharide).

Administration of Polysaccharide in dose of 15 mg.

30 minutes after injection, there was as yet no manifestation of the inhibitory activity, although leucocytotic reaction was most active in this stage, degenerated monocytes being noticed occasionally. As to the picture of cell division, the chromosomes were observed distinctly, their number ranging between 30 and 40. Relative to the leucocytes, number of the neutrophils increased steadily, reached maximum nearly at the 3rd hour, but began to fall around the 16th hour with concomitant cellular degeneration. Tumor cells, as stained supravitally, were of usual size, the nucleoli being large and stained distinctly, mitochondria in usual number and azure granules in normal distribution in the paranuclear area. One hour after injection, the cells showed a picture of slight nuclear deformity, atrophy and thickening of nucleoli, although pronounced swelling of cytoplasm was encountered occasionally, being several times of the usual size. The alteration of the paranuclear area of cytoplasm, particularly where there was rich distribution of azure granules, was outstanding in that it was initiated by small vacuole formation. It seemed that dysfunction occurred first in Golgi apparatus. Cells appeared generally hypertrophic, and showed an increased basophility, with nuclei nearly compact. Along with the steady increase of leukocytic reaction, migration of macrophages occurred, but it subsided by the 24th hour to restore tumor-cells to the previous state of active proliferation. The lesions of the cytoplasm remained for as long as 6 hours, but the growth of tumor cells was resumed thereafter, the complete resumption being accomplished by the 24th hour.

Administration of Polysaccharide in dose of 40 mg.

Specimens of the ascites, in the state of pure culture of tumor cells prior to injection, showed the picture of leukocyte reaction 30 minutes to 1 hours after the injection, and the specimens were loaded with numerous leukocytes, two several times as many as tumor cells, where every one tumor cell was found surrounded

by a mass of leukocytes. Although no marked change was yet produced in tumor cells in this stage, cytoplasm swelled up markedly after the 3rd to 6th hour and looked hyaline-transparent, probably due to a decreased viscosity of the cytoplasm, and this gave an impression as if plasm-membrane has been distended. Increased number of tumor cells showed the picture of perinuclear vacuole formation. It seemed that polysaccharide caused the tumor cells to be slowly degenerated centripetally from the periphery. It was found in every instance that the cytoplasm was the only part invoived in the degeneration, and the nuclei and nucleoli remained intact, and so did the chromosome in the metaphase and anaphase of the cell division. The cytological manifestation of the tumor-inhibitory activity was similar between polysaccharide (in the dose of 40 mg) and nitrogen mustard in many aspects, except for the difference that the former affected the cytoplasm while the latter affected the nucleus. Besides this, a marked difference arose in connection with how fast the inhibitory effect came into existence; namely, the severity of the changes 30 minutes after the administration of nitrogen mustard was equivalent with that resulting 3 to 4 hours after polysaccharide injection.

Administration of Polysaccharide in the dose of 60 mg.

The outstanding feature was the specific destruction of the resting cells, and the cells in mitosis seemed to be free from any pronounced primary lesion. Namely, the changes of resting cells were initiated with such patterns of lesion as plasmolysis, hyaline swelling of cytoplasm, standing of nuclei out of softened cytoplasm. Along with these changes, atrophy and destruction occurred in the leukocytes, which were partaking in the already active leukocytosis. Six hours after injection, the leukocytic reaction including neutrophilia subsided, the perinuclear vacuole formation as well as lysis of cytoplasm being the outstanding picture. In case further destruction proceeded, this picture was modified by the presense of naked nucleus similar to the one caused by Nitromin or Colchichin administration. However, nuclei were not involved whatever in the development of cytoplasmic degeneration. Some of the cells with highly degenerated, softened cytoplasm were hardly stained by the dyes and looked hyaline transparent, although they were seldom subject to such type of degeneration as formation of droplet-sized vacuoles. These two types of degenerated cells showed none of the solid structure such as neutrophil granules, Golgi apparatus, mitochondria or metachondria.

Consideration

It has been demonstrated that the tumor inhibitory activity of polysaccharide was limited within the scope of cytoplasm, and seldom involved nuclei. Polysaccharide failed to meet the necessary condition for a cancer-inhibitory agent that it interferes with the cell division and growth. The characteristic feature of the activity of polysaccharide was that it induced an alteration specifically in the cytoplasm of

tumor cell. The fact that no preliminary knowledge was available as to the adequate dosage, together with the fact that only few animals were studied for the effect of successive administrations hindered the authors greatly from reaching more solid results. Since not only the nucleus-attacking agent, but also the cytoplasm-poison as well may serve as the anti-carcinoma agent, and since fairly marked lesion was actually produced in the cytoplasm of tumor cells in the above experiment, although slowely in its onset and transient in its duration, it may well be expected that someday we may give polysaccharide to tumor-bearing subjects in an improved manner with gratifying result. The synthesis of polysaccharide being possible today, it may be duly hoped that more powerful compound will be synthesized by substituting the active radicals with those of higher activity. It may be possible, too, to duplicate the effect of polysaccharide by covering the drawbacks of the present method by simultaneous administration of other drugs. It deserves attention that the polysaccharide elavolated by the tumor tissue seems to be injurious to the growth of the same tumor. There is a need of further investigation on this subject, particularly on the possibility of DNA synthesis in the sarcoma tissue, because, in case such synthesis occurs in the sarcoma tissue, effect of polysaccharide on DNA synthesis should be a subject of fruitful research.

Conclusions

(1) Specimens of polysaccharide, obtained by fractionation at low temperature from a mass of the subcutaneous growth of Yoshida sarcoma, act specifically on the cytoplasm of Yoshida ascites tumor cells. The earliest manifestation of the cytoplasmic lesion takes place in the paranuclear area.

(2) Average yield of polysaccharide is 1.104 mg per gram of the tumor tissue.

(3) Administration of polysaccharide is innoxious to the living body. As judged from the results of parallel experiments with different doses, varying as 15, 40 and 60 mg, tumor-inhibitory effect was proportionate to the dose administered, but was transient irrespective of the doses.

Acknowledgements

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Explanation of Plate XXIII

(1) Administration in dose of 15 mg.

Fig. 1, Fig. 2. It is obvious from both photographs that the initial vacuoles formation takes place at the paranuclear area. No abnormality occurs in the mitosis.

Fig. 3, Fig. 4. Polysaccharide attacks the most active part in metabolism and leads to the degeneration associated with vacuoles formation.

Plate XXIV

(2) Administration in dose of 40 mg.

Fig. 5. 30 minutes: Appearance of vacuoles.

Fig. 6. 1 hour: Perinuclear vacuole formation.

(3) Administration in dose of 60 mg.

Fig. 7. 4 hours later: The picture indicates the lysis and disappearance of cytoplasm.

Fig. 8. 6 hours later: Diminished size of cytoplasm as compared with the size of the nucleus.

要　旨

多糖類の吉田肉腫細胞に及ぼす影響（予報）

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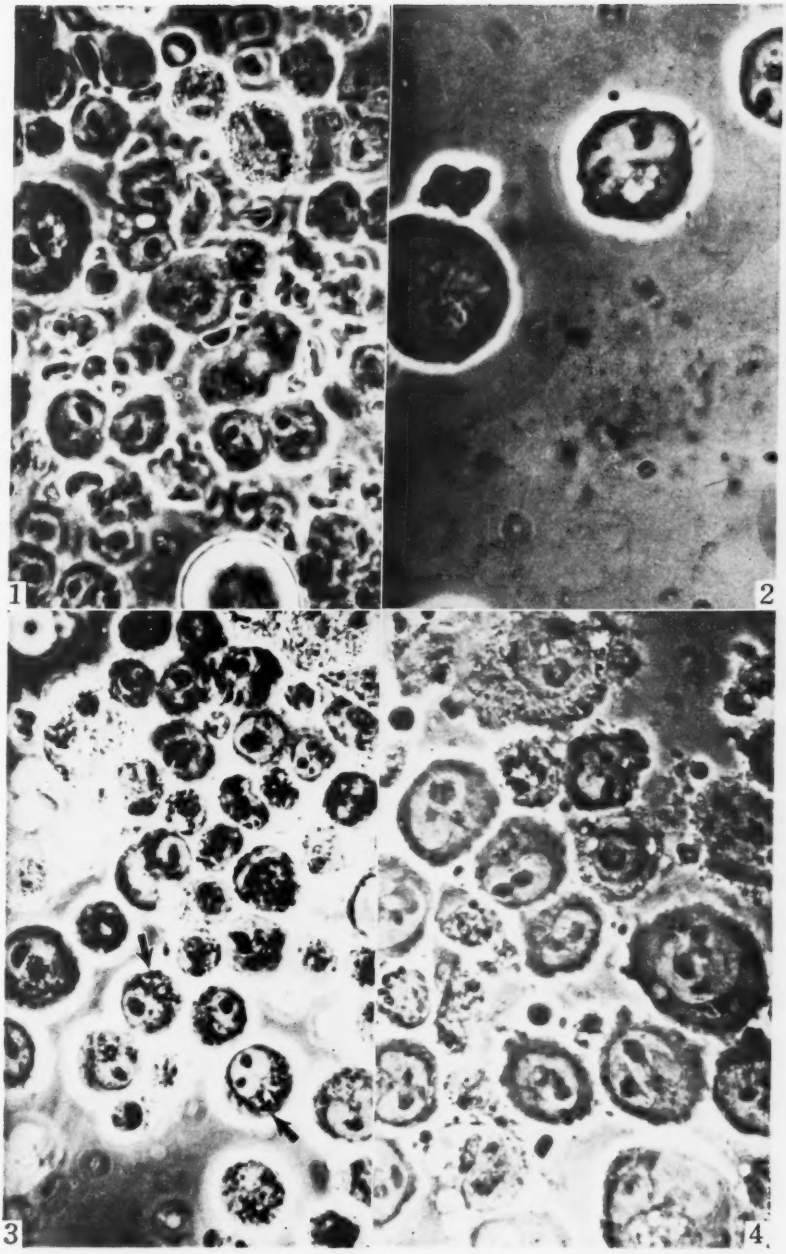
II 及び III Capsuläre Pneumokokken の型特異性多糖類を抽出してその特異性免疫反応について研究中，その中和抗体機序に興味ある事実を認めた事よりヒントを得，この理を吉田肉腫細胞に応用してみたならば如何なる結果を得るだろうかと，肉腫中多糖類を低温下に抽出しその影響を観察したところ若干の知見を得たので記述した。しかし現在なお，持続投与例並びに追加試験を継続中であり未結論のままであるが，得た所見を予報として記載した。肉腫多糖類は醋酸反応とクロロフォルム，ブタノール抽出法を利用し，アルコール沈澱をもって得，反復抽出を施して精製品を得た。吉田肉腫細胞に及ぼす影響は，純培養状態にて直接腹腔内に食塩水にて溶解した多糖類を注入して腹水細胞に接解させた直接効果の有無を時間を追い検討した。肉腫細胞原形質に選択的に融解作用を及ぼし，この際，原形質のいわゆる明庭部に最も早く障碍を引き起すことを知った。核に対する直接作用は全く認められなかったが，60 mg の大量投与の際には，原形質が完全に融解消失して裸核像を呈するに到る細胞もあった。しかし腫瘍抑制効果としては作用は一時的で永続性に欠けるが，他の抑制物質（例えば Nitrogen Masturd や Colchichin）に較べ生体に及ぼす害の比較的少い利点があり，その連続投与効果について目下研究中である。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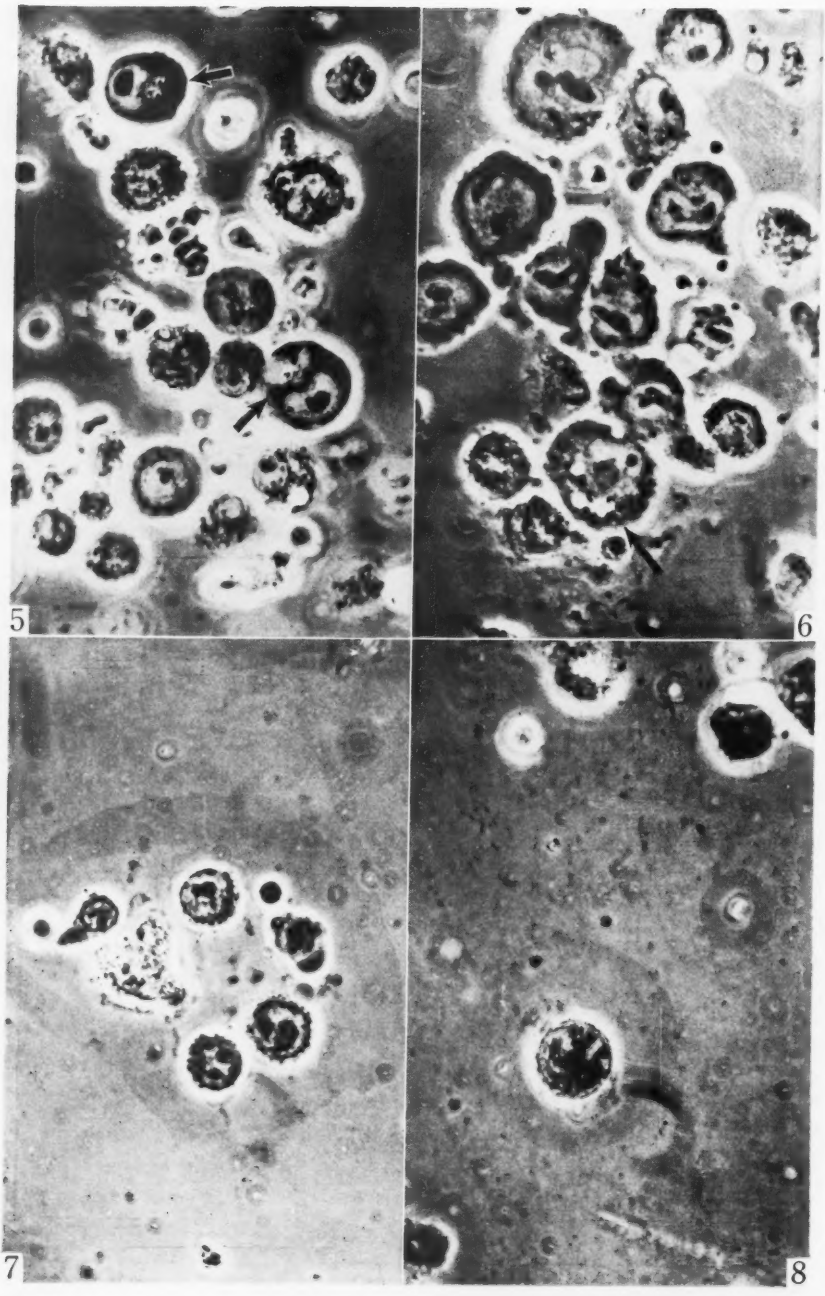
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[GANN, Vol. 44; December, 1953]

DISTRIBUTION OF TUMORS IN VARIOUS ORGANS FOLLOWING THE TRANSPLANTATION INTO THE LEFT CHAMBER OF THE HEART. (STUDIES WITH THE YOSHIDA SARCOMA AND THE ASCITES HEPATOMA)

(With Plates XXV-XXVII)

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In the case of malignant tumor metastasis, it is a well-known fact that certain tumors have generally some particular sites to which they are very likely to metastasize. Liver metastasis of cancers in the area of the portal system or lung metastasis in general may be fully understood by the mechanical fact that the tumor emboli are apt to occur in the capillaries of these organs. But the fact that cancers of the thyroid and the prostate glands are apt to metastasize in the bones is not to be explained by mere mechanical reasons. Some investigators have made experimental studies of the question. The best method for such experiment may be to inject tumor cells into the left chamber of the heart (into the blood) so that they may be distributed to every part of the body, and to observe whether the formation of metastasis varies according to organs or not. In 1949 Dale R. Coman and his cooperators[1] made an experiment of the transplantation of V2 carcinoma into the left chamber of the heart, and reported the states of formation of tumors in various organs. And again in 1951 they made similar experiments using the Brown-Pearce domestic rabbit tumor[2].

According to them, frequency of tumor metastases in some organs or scarcity of them in others depends upon the mechanical factor whether tumor emboli are very liable to get to the organ, especially to its capillaries; and the organ's susceptibility to tumors plays only the smallest part in the occurrence of secondary tumor.

Their experiments were made by using the finest emulsion as could be made of nodulous tumor, taking the form of as many separate cells as possible.

The Yoshida sarcoma is the sarcoma of perfectly separated cells. The ascites heptoma[3] produced in our laboratory is an ascites tumor having many pairs of cells and many 'islands', (groups of a small number of cells). These transplantable tumors are the most suitable materials for the research now in question. Accordingly, with these materials I was engaged in the study concerned.

It may be said in this connection that in 1951 Tagashira and some others[4] reported that they transplanted the Yoshida sarcoma into the femoral vein and found

the high degree of tumor formation only in lymph nodes and bone marrow, noticing not so remarkable increase of tumors in other organs.

I. Experiment of Transplantation of the Yoshida Sarcoma into the Left chamber of the Heart

1. Materials and Methods

(A) 0.03–0.05 cc of the tumor ascites of the Yoshida sarcoma (including about 10,000,000 supposed tumor cells) diluted five times with physiologic NaCl-solution was directly injected into the left chamber of the heart of the rat weighing 100 g or there about.

That the tip of the injection needle successfully enters the cavity of the left ventricle is almost made sure by observing that the color of the blood flowing backward into the injector is bright red and that the inside cylinder of the injector is rhythmically pushed upward in accordance with the pulse. It is as yet difficult to decide whether the place is the left strium or the left ventricle. But it can be justly said that the ascites could be transplanted into the left chamber of the heart.

More than one week after the transplantation were examined 29 cases (15♂+14♀) which died from tumor, and were investigated the state of increase of tumor cells in the peripheral blood, the ascites, the pleural fluid, and the pericardial fluid, and the state of formation of tumors in the following 31 organs: brain, eyes, lacrimal glands, heart, lungs, thymus gland, spleen, kidneys, adrenals, liver, pancreas, stomach, intestines, bladder, testicles, epididymis, prostate, seminal vesicles, ovaries, uterus, tongue, parotid glands, submaxillary glands, sublingual glands, esophagus, thyroid glands, aorta, bone marrow, skeletal muscles and skin.

(B) 1 cc of the tumor ascites (including more than 300,000,000 supposed tumor cells) diluted twice with physiologic NaCl-solution was injected into the left chamber of the heart. The animals multitudinously transplanted with the ascites of this sort die several minutes after the transplantation. Two of such cases were investigated as to the state of distribution of tumor cells in every organ immediately after the transplantation.

Additional remarks:

The sudden death shortly after the transplantation was rarely observed. When it happened, it was chiefly due to the formation of hematopericardium caused by the damage of coronal artery in the transplantation.

In this experiment 3 negative transplantation cases out of the 32 cases, excepting the accidental death, were observed. In addition, the experiment of transplantation into the left chamber of the heart was attempted on the 5 cases which had proved negative in the intraperitoneal transplantation, all of which again proved

negative. These additional experiments were made to know whether the cases which had proved negative in the intraperitoneal transplantation could be positive in some organs of not after transplantation into the left chamber of the heart, but the formation of tumors was not observed in any organ of each of the 5 cases.

2. Results

(A) The average number of days of existence of the 29 positive cases is 10 days (6-20), making no great difference from that of intraperitoneally transplanted cases.

In 3 cases the peripheral blood from the tail was examined directly after the injection into the left chamber of the heart (within one minute after), but no tumor cells could be found there, while several days later tumor cells were found more easily in the peripheral blood. In the last stage tumor cells were found out almost always in the peripheral blood, and the largest number of the cells observed amounted to 12 % of all the leucocytes.

Intraperitoneal transplantations of the peripheral blood (about 0.01 cc) of animals transplanted into the left heart were tried directly after (within one minute), two days after and five days after the transplantation into the left chamber of the heart. Among these, 1 out of 3 directly after cases, 2 out of 6 two-days cases and 5 out of 8 five-days cases proved successful. This shows that even when the transplantation took place in the blood, very few tumor cells remain in the blood directly after the transplantation and they increase little by little day after day until they were largest in the end. In other words it may well be considered that transplanted cells scarcely circulate themselves through the flowing blood and that they are not transfered to the flowing blood until the cells lodged at some part of the organ increase in number and that they gradually keep on growing in this way.

In 21 out of 29 cases tumor cells appeared in the ascites 9 days after the transplantation on the average (earliest 8 days, latest 12 days), but did not reach the state of the pure culture even just before the death. However, in the pleural fluid and the pericardial fluid the state of pure culture of tumor cells was seen one week after the transplantation on the average. The thickening of the pericardium and the pleura due to tumor was observed at autopsy.

There exists a certain recognizable difference among the degrees of tumor increase in the ascites, in the pleural fluid and in the pericardial fluid. It may depend on the circumstances that tumor cells can get into the peritoneal cavity only hematogenously, while they are often directly put into the pericardial cavity and the pleural cavity during the transplantation.

Organs producing macroscopical tumors among various organs, and the percentages of those tumor appearances are summarized in Table 1.

Table 1. Distribution of macroscopical metastases in 29 rats inoculated with Yoshida sarcoma cells into the left chamber of the heart.

Organ	Number of animals in which macroscopical metastases were found			
	(A)	(B)	(C)	Total
Lymph nodes			29	29
Kidneys	13	12		25
Heart	24			24
Adrenals		5	16	21
Thymus glands		6	12	18
Intestines	2	13		15
Stomach		12		12
Lungs		8		8
Liver		4		4
Pancreas	2			2
Thyroid glands			2/10	2/10
Ovaries	•		9/14	9/14
Uterus	3/14			2/14

(A): Metastases were observed as tumors on the surface of the organ.
(B): Metastases were observed as very small hemorrhagic spots.
(C): Metastases were observed as the enlargement of the whole organ.
Microscopically, in lungs, liver, and pancreas, conspicuous increase of tumor cells were often observed.
Thyroid glands were examined only in 10 rats, and ovaries and uterus in 14 female rats.

Heart—Multitudinous occurrence of hemispherical tumors is always observed on the surface of the organ. The increase of tumor cells within the myocardium is also observed microscopically.

Kidneys—In the cases survived more than 10 days innumerable small hemispherical tumors were confirmed almost always on the surface of the kidney, while in the cases existing within 10 days were hemorrhagic specks, where the increase of tumor cells was microscopically perceived, occurred evenly on the surface of both kidneys.

The finding that tumor nodules occurred multitudinously and evenly on the surface of both kidneys is most conspicuous of all the findings of the organs when they were affected by the transplantation of tumor cells into the left chamber of the heart.

Lungs—Many tumors occurred on the surface of the organ as semitransparent greyish white specks, but they never caused prominence as swelling tumors. Even

in the case in which the macroscopical findings was not apparent the increase of tumor cells was almost always observed microscopically in every portion of all the lobes.

Liver—In the 4 cases of comparatively anemic livers, darkbrown hemorrhagic specks of small round type or of irregular type were observed on the lower surface of the liver, and it was microscopically ascertained that they were the intensely infiltrated part of tumor cells. In other cases tumor cells were almost always increasing diffusely in the intralobular sinusoid as well as in the Glissonian capsule. In one case (8 days after the transplantation) was observed a localized tumor measuring 2 mm in diameter around the bile duct. In general, however, macroscopical tumors were scarcely formed in the liver.

Pancreas—Macroscopical nodular tumors were observed only in 2 cases, but it was not rare that the increase of tumor cells was microscopically observed.

Stomach—Hemorrhagic specks were observed on its serous side: and its mucous side showed always cancerous ulcers. It occurred always in the lower half of the stomach provided with the mucous membrane, and never occurred in the forestomach having the structure similar to that of esophagus.

Intestines—The same macroscopical finding as described in the stomach was recognized in this organ. It is noticeable that the duodenum is the most favorable seat for the tumor to occur, while it sometimes occurs in all parts of the small intestines. However, it occurs very seldom in the large intestine.

In 2 cases, one existing for 19 days and the other for 20 days, the formation of bead-like enlarged tumors of highly multitudinous occurrence was observed in the duodenum, and the same high degree of tumors were found scattered in the jejunum (Fig. 1). It was noticed in these two cases that no remarkable tumor was observed in any other organ.

Adrenals—The whole organ swelled and showed dark red specks on its surface. Histologically the border line between the cortex and the medulla could not be defined in this case, and the whole organ was infiltrated with tumor cells.

Thyroid glands—The whole organ swelled slightly. It showed dark red specks on the surface, which is the same phenomenon as was observed in the case of the adrenals.

Ovaries—The whole organ swelled to such a great extent that the original structure of the organ could not be identified even microscopically at all. This is the same finding as in the adrenals (Fig. 2).

Uterus—In 2 cases out of 14 females were observed bead-like nodes. The uterus did not swell as a whole, but in several swelling parts of the organ the spherical thickening was shown (Fig. 2).

Thymus gland—The whole organ swelled remarkably. The increase of tumor cells was observed in such a degree that the original structure could not be

histologically identified at all.

Lymph nodes—Tumors were observed as an enlargement of the whole organ, but it was conspicuously observed mainly in the cervical, the paratracheal, and the mesenteric lymph nodes. The swelling of lymph nodes was generally not so conspicuous as in the ascites hepatoma as will be mentioned below.

In organs indicated in Table 2 no evident macroscopical formation of tumors was observed, but histologically tumor cells were found in some of them. Histological findings are summarized in Table 2.

Table 2.

Distribution of microscopical metastases in those organs in which macroscopical metastases could not be found (10 rats inoculated with Yoshida sarcoma cells into the left chamber of the heart).

Number of animals in which microscopical metastases were found			
Bone marrow	8	Parotid glands	0
Spleen	7	Submaxillary glands	0
Skeletal muscles	2	Tongue	0
Brain	2/15*	Esophagus	0
Seminal vesicles	2	Aorta	0
Prostate	2	Bladder	0
Sublingual glands	1	Skin	0
Eyes	0	Testicles	0/15
Lacrimal glands	0	Epididymis	0/15

In the bone marrow, prostate and seminal vesicles, the increase of tumor cells was often conspicuous, but not in the other organs.

* Brain, testicles and epididymis were investigated in 15 rats.

In 15 cases thorough histological examination of the brains was done. In 2 cases out of them was found out poor increase of tumor cells localized in the plexus chorioideus, but in no other place.

In one of these two cases more than half of the tumor cells were found in the necrotic state.

Spleen—In 3 cases out of 39 swelling of the spleen was observed at the last period. In these 3 cases diffuse increase of tumor cells was confirmed. In other cases the increase of tumor cells was not so conspicuous, or could not be exactly identified.

Bone marrow—The formation of the tumor could not be definitely observed macroscopically, but in almost all the cases a high degree of diffuse increase of tumor cells was microscopically perceived.

Skeletal muscles—In all the cases the formation of the tumor could not be observed macroscopically, but in 2 cases out of 10, in which the thigh muscles were microscopically investigated, was observed the localized increase of tumor cells.

Sublingual glands—10 cases were microscopically examined. In 1 case out of those examined was observed a slight degree of increase of tumor cells.

Prostate—10 cases were microscopically examined. In 2 cases out of those examined was observed a comparatively high degree of increase of tumor cells.

Seminal vesicles—In the 2 cases forming the microscopical tumor in the prostate was also observed a high degree of increase of tumor cells.

No one case in which tumor cells were evidently identified in the histological examination ever existed in each set of 10 cases of the following organs: Eyes, Lacrimal glands, Parotid glands, Submaxillary glands, Tongue, Esophagus, Aorta, Bladder, Testicles, Epididymis, Skin. (In eyes, testicles and epididymis, however, 15 cases were examined.)

As a dot-like hemorrhagic speck was perceived at the inside of the cornea in 1 case of the eye, a serial section was made use of in the investigation, but no tumor cell was found out.

As a hemorrhagic speck localized on the surface was observed in 1 case of the testicle also, this part was examined with a serial section, only to find no tumor cell.

(B) In order to investigate the distribution figure of tumor cells in various organs directly after the transplantation of the tumor into the left chamber of the heart following two experiments were done:

At first, about 10,000,000 tumor cells were transplanted and the state of distribution of tumor cells was examined, but no tumor cells could be found out in the testicles, brains and eyes.

Then, about 300,000,000 cells were transplanted into two rats. Both animals died several minutes after the transplantation. In these animals tumor cells could be found in the above mentioned three organs: As to the brains, tumor cells were perceived in the bloodvessels of the plexus chorioideus; as to the testicles, in the interstitial capillaries. In all other organs tumor cells were found very easily. But in those organs as spleen, bone marrow or parotid glands some difficulty of identification of tumor cells was experienced.

II. Transplantation of the Ascites Hepatoma into the Left chamber of the Heart

1. Materials and Methods

(A) The method of transplantation in this experiment is the same as is adopted in that of the Yoshida Sarcoma. About 0.05 cc of the tumor ascites (about one week old), diluted five times with physiologic NaCl-solution, was transplanted into the left chamber of the heart. In 22 cases (♂11+♀11) which died on tumor development in various organs after the transplantation into the left chamber of the heart

the same investigation as that on the Yoshida sarcoma was made.

(B) In order to investigate the distribution of tumor cells directly after the transplantation a large number of tumor cells was transplanted into 2 animals, that is to say, 0.3 cc and 1.0 cc of the tumor ascites (diluted 2 times with physiologic NaCl-solution) were transplanted respectively and the animals died almost instantly after the transplantation, the state of distribution of tumor cells in each of the organs was investigated.

Additional remarks:

In this experiment far more animals than in the experiment on the Yoshida sarcoma died directly following the transplantation. This was chiefly brought about by the tumor emboli in the coronal artery of the heart (Fig. 3). The principal cause of this phenomenon is that in the case of Yoshida sarcoma the individual tumor cells are completely free, while the cells of the ascites hepatoma form at least the pair of two cells, usually groups of several cells or islands. As there existed a fairly large group of the cells in the ascites hepatoma, it was attempted in this experiment to use tumor ascites containing as small islands as possible. However, even with such material, when 0.1 cc was introduced about 50% animals died immediately following the transplantation. Then, the amount was reduced to 0.05 cc with the result that almost all animals could tolerate the transplantation.

2. Results

The average days of survival of the 22 successfully transplanted cases were 13 (8–22), indicating that in this case they were rather shortened than in the intraperitoneal transplantation of the same tumor.

The capillary blood (in the tail) taken after the transplantation was investigated in more than half of all the cases experimented immediately after and several days after the transplantation, and at the last period of tumor growth. As the result there was no one case in which tumor cells on the smears were apparently identified. This is a remarkable difference compared with that of the Yoshida sarcoma.

As to the tumor increase in the peritoneal cavity, only in one case out of 22 tumor cells were recognized in the ascites at late stage of tumor growth in other organs of its body. However, in the pleural fluid and the pericardial fluid, the increase of tumor cells was, just like in the Yoshida sarcoma, very frequent.

The state and frequency of tumor formation in various organs are summarized in Table 3.

Brain—The macroscopical enlargement of the plexus chorioideus from tumor was seen in almost all the cases (Fig. 4). Furthermore, in 6 cases among them some hemorrhagic specks were observed macroscopically on the surface and on the section of the brain. However, in the investigation immediately after the transplan-

Table 3.

Distribution of macroscopical metastases in 22 rats inoculated with the ascites hepatoma cells into the left chamber of the heart.

Organ	Number of animals in which macroscopical metastases were found			
	(A)	(B)	(C)	Total
Lymph nodes			22	22
Brain (plexus chorioideus)			22	22
Eyes (anterior chamber)		4	18	22
Heart	18			18
Adrenals			17	17
Lungs	6			6
Kidneys	3			3
Pancreas	3			3
Skeletal muscles	3			3
Bone marrow		2		2
Skin		2		2
Spleen		1		1
Liver	1			1
Thyroid glands	1			1
Thymus glands		1		1
Ovaries			8/11	8/11
Testicles		1/11		1/11

(A), (B), (C): the same as in Table 1.

tation no difference was noticed in distribution of tumor cells between the parenchyma and the plexus chorioideus.

Eyes—It is noteworthy that in all animals transplanted the tumor cells increased in the eye-ball. Already five days after the transplantation small turbid whitish or hemorrhagic specks were observed on the corneal margin, which grew very rapidly.

At the last period the eye-ball swelled and projected conspicuously (Fig. 6). The aqueous humour of the anterior chamber turned quite turbid and thus the power of vision came to be completely lost. Microscopically the anterior chamber became to the state of the pure culture of tumor cells, and the ciliary body and the iris were most severely invaded by the tumor cells, so that their original structure

could not often be identified (Fig. 5).

However, the increase of tumor cells in the choroidea was very rare. In all cases no invasion of tumor cells was found in the cornea, the sclera, the vitreous body and the retina.

In the investigation of the eye-ball directly after the transplantation tumor cells were distributed not only in those tissues as iris and the ciliary body, but also in the capillary of the retina tumor cells could be identified in one case. But, even in progressed stages tumor increase in the retina was found in no case.

Heart—The same as in the Yoshida sarcoma.

Kidneys—Although the kidneys were the organ in which the tumors were very apt to develop in the case of the Yoshida sarcoma, they formed very few tumors either macroscopically or microscopically in the case of the ascites hepatoma. However, in 3 cases out of 22 small scattered nodules were observed on the surface. Histologically, an increase of tumor cells localized in the glomeruli were rarely observed. In the investigation immediately after the transplantation, tumor cells were found mainly in the smallest arteries of the cortex, but very few or none in the glomeruli (Fig. 7).

Lungs—Only in few cases small nodules were scattered on the surface of the lungs. But, tumor formation in the lungs turned out abruptly to be of a great extent when the tumor ascites was transplanted into the right chamber of the heart (4 cases) or into the femoral vein (3 cases). In these cases the lungs lost their original shape, being covered with piles of tumor nodules (Fig. 8), while hardly any tumors were found in any other organ, except in the lymph nodes belonging to and related to the lungs (the paratracheal, the mediastinal and the cervical) and the retroperitoneal lymph nodes. But only in 1 case of the transplantation into the femoral vein some small tumor nodules were observed in the plexus chorioideus of the brain, kidneys, and adrenals. In the transplantation either into the right chamber of the heart or into the femoral vein the days of survival of tumor animals were considerably (on the average 8 days) prolonged compared with those of the transplantation into the left chamber of the heart. The survival days of the right-side transplantation cases were 21 days on the average (19–23 days).

Liver—Even in cases examined immediately after the transplantation of a large amount of tumor ascites, e. i. 0.3 cc (6 times as much as usual cases of this study) no tumor cells could be found in the liver, while in other organs of the same cases, for example in the brain, eye-ball, stomach, kidney, testicle, etc., tumor cells were distinctly identified. But in the cases transplanted with 1 cc tumor ascites (20 times as much as usual ceses) some tumor cells, examined directly after the transplantation, were clearly identified in the arterioles of the Glissonian capsule (the branches of hepatic artery) or in the central vein. As to the advanced stages of 0.05 cc transplantation, only in 1 out of 22 cases the increase of tumor cells

localized in the central vein was histologically observed. However, many of the cells of this case were degenerative.

Pancreas—The same as in the Yoshida sarcoma.

Adrenals—The organs swelled far larger than in the cases of the Yoshida sarcoma. (Fig. 9) Histologically the medulla completely lost their original structure and were thoroughly infiltrated with tumor cells, while the cortex (especially its margine) was scarcely affected, becoming in the state of wrapping the tumors. Nevertheless, in the examination directly after the transplantation the tumor cells could be found only in the subcapsular capillaries of the adrenals.

Testicles—In only 1 out of 11 cases a subcapsularly localized hemorrhagic tumor was macroscopically found. It was shown that the tumor cells increased only in the interstitial tissue without showing any destructive infiltration into the parenchyma.

Ovaries—The same as in the Yoshida sarcoma, but the degree of swelling of the whole organ is far higher.

Histologically the original structure could not be identified very often.

Spleen—In 1 out of 22 cases multiple macroscopical greyish white specks were found throughout the enlarged spleen tissue on the 5th day of the transplantation. Histologically it was confirmed that the tumor cells were increasing. In another case (12 days after the transplantation) a large amount of tumor cells were evidently identified scattering throughout the remarkably enlarged spleen, though the formation of localized tumor nodules was not conspicuous. Only in these two cases the increase of tumor cells was confirmed, whereas immediately after the transplantation of large amount of tumor cells, they were distinctly observed both in the trabecular arterioles and in the sinusoids.

Bone marrow—Conspicuous hemorrhagic specks were observed in the femur of 2 cases indicating a high degree of increase of tumor cells. In the same two animals it was observed that the successive tumor swellings occurred in the borders of the bone and the cartilage of all the ribs on both sides (Fig. 10) They appeared just like the rachitic rosary. Directly after the transplantation an exact identification of the tumor cell in the bone marrow was very difficult.

Skeletal muscles—It was often very easy to find tumor cells in the muscles immediately after the transplantation, but generally a very few tumors were formed there in advanced stages. In 3 cases, however, macroscopical tumor nodules were formed almost all over the body: especially noticeable in the trunk, the thigh and the shoulder (Fig. 11). In 1 case among them many conspicuous tumor nodes occurred in the diaphragm.

Skin—In 2 cases numerous hemorrhagic tumor nodules increasing in the cutis were observed, especially conspicuous in the skin on the back (Fig. 12).

Thymus gland—Compared with the case of the Yoshida sarcoma, very few

metastases were found in the case of the ascites hepatoma. Even when many lymph nodes surrounding it swelled to a great extent, the thymus gland remained always unaffected. In 1 case some hemorrhagic specks were observed; and histologically merely an extremely slight degree of increase of tumor cells was observed.

Lymph nodes—The grade of enlargement of the lymph nodes by the tumor increase was more remarkable than that in the Yoshida sarcoma. But, macroscopically, the estimated rate of the occurrence of the lymph nodes metastases differed considerably by the location as indicated in Table 4.

Table 4. Distribution of lymph-node-tumors in various sites in 22 rats inoculated with the ascites hepatoma cells into the left chamber of the heart.

Site	Number of animals in which macroscopical metastases were observed
Retroperitoneal	22
Posterior mediastinal	22
Paratracheal	22
Cervical	18
Mesenteric	8
Subcutaneous (nape)	8
Axillar	6
Subcutaneous (in other site than the nape)	5
Inguinal	5

Among the lymph nodes the retroperitoneal ones (especially around the kidneys) showed sometimes a remarkable enlargement. In such cases the adrenals also swelled to a high degree, but the kidneys remained unaffected (Fig. 13). In the examination immediately after the transplantation, some tumor cells were found in the arterioles around, and also inside of the capsule. It might be noticeable that all these findings demonstrated clearly that the wide-spread lymph nodes metastasis occurred by the way of the blood stream, and that the lymph nodes metastasis of the hepatoma (carcinoma) was more remarkable than that of the sarcoma.

Table 5. Distribution of microscopical metastases in those organs in which macroscopical metastases could not be found. (10 rats inoculated with the ascites hepatoma cells into the left chamber of the heart.)

Number of animal in which metastases were observed microscopically			
Lacrimal glands	1	Epididymis	0
Sublingual glands	1	Parotid glands	0
Esophagus	1	Tongue	0
Prostate	1	Submaxillary glands	0
Stomach	0	Aorta	0
Intestines	0	Bladder	0
Uterus	0	Seminal vesicle	0

Thyroid glands—In only 1 case small tumor nodules occurred multitudinously, without showing a swelling of the whole gland.

In other organs than described above no tumor formation was found macroscopically. But microscopically, the increase of tumor cells of various degrees was confirmed in some of them. The findings are summarized in Table 5.

Among the organs indicated in Table 5, it may be noted that in 7 organs, namely epididymis, parotid gland, tongue, submaxillary gland, aorta, bladder and seminal vesicle tumor cells could not be found even in the examinations immediately after the transplantation of a large amount of tumor cells. In such organs as stomach, intestine and uterus tumor cells were evidently distributed in the examinations immediately after the transplantation, but they did not show any increase in these organs. On the contrary, in sublingual gland, esophagus and prostate tumor cells were found only in advanced stages. Compared with the Yoshida sarcoma the lack of tumor formations in the stomach and intestine may be noteworthy.

Comparison of the Yoshida Sarcoma with the Ascites Hepatoma

It is tried to discuss the differences of the metastasis formation, which were demonstrated in the experiments between the Yoshida sarcoma and the ascites hepatoma. Results are summarized in the Table 6.

(A) Remarkable differences may be found in following organs:

1) Eye—In all cases of the ascites hepatoma the tumor formation in the eye-ball was noted, and that almost always in a high degree, while in the cases of the Yoshida sarcoma tumor formation in the eye was never observed.

Metastasis of carcinoma in the eye-ball is not rare in human cases. More than 200 cases of that sort have already been reported.[5,7,8] But only a few cases are reported in our country amounting to less than 20. It is reported that, so far as man is concerned, the location where the metastasis takes place is almost always limitted to the choroidea, and occurs most frequently in the extremely back part of the eye-ball. As for the primary tumor the mammary carcinoma is overwhelming, occupying 2/3 of 192 cases of carcinoma which metastasized in the eye.[7]

The metastasis of sarcoma in the eye-ball of the human being is a very rare case.[5,6,9] A case has never been reported in which the metastasis of sarcoma from a distant organ has ever occured. Several cases reported are limitted to the successive metastases from the adjacent organs or that from the other eye.

The results of my experiments are, therefore, exactly corresponding to the observations in human cases. But, in human cases the location of metastasis is mainly the choroidea, while in the cases of my experiment it is limitted to the anterior chamber, the iris and the ciliary body. The frequent occurrence of metastasis in the anterior chamber in animal cases are also observed by Coman

Table 6.

Comparison of the Yoshida sarcoma with the ascites hepatoma in the frequency of the formation of tumor in various organs following the transplantation of the tumor cells into the left chamber of the heart.

Organ	Yoshida Sarcoma	Ascites Hepatoma	Organ	Yoshida Sarcoma	Ascites Hepatoma
Lymph nodes*	###	###	Sublingual glands	+	+
Heart	###	###	Prostate	+	+
Lungs	###	##	Seminal vesicles	+	−
Kidneys	##	+	Uterus	+	−
Bone marrow	##	+	Eyes	−	###
Liver	###	+	Skin	−	+
Adrenals	##	##	Lacrimal glands	−	+
Ovaries	##	##	Testicles	−	+
Pancreas	##	##	Esophagus	−	+
Thymus	##	+	Epididymis	−	−
Spleen	##	+	Parotid glands	−	−
Stomach	##	−	Submaxillary glands	−	−
Intestines	##	−	Tongue	−	−
Brain	+	###	Bladder	−	−
Skeletal muscles	+	+	Aorta	−	−
Thyroid glands	+	+			

###: Tumors were found in more than 8 out of 10 rats macroscopically or microscopically.

##: Tumors were found in more than 4 out of 10 rats macroscopically or microscopically.

+: Tumors were found in less than 2 out of 10 rats macroscopically or microscopically.

−: No tumor was found in any case experimented.

In the case of lymph nodes, the frequency of tumor appearance is almost equal in both cases, but the degree of tumor enlargement is far more conspicuous in the cases of the ascites hepatoma than in those of the Yoshida sarcoma.

and his co-workers.[1,2]

2. Brain—As for the ascites hepatoma, noticeable tumor formations in the plexus chorioideus were observed in almost all the cases, while in the cases of the Yoshida sarcoma the increase of tumor cells in the same place, which was observed in a few cases, was very weak.

3. Stomach and Intestines—In the transplantation of the Yoshida sarcoma, the metastasis occurred most frequently in the stomach and in the duodenum (some-

times occurring in other parts of the small intestine), while, in that of the ascites hepatoma, no formation of tumors was observed in these parts.

4. Kidneys, Liver, Bone marrow, Thymus—In the transplantation of the Yoshida sarcoma, tumors were very likely to occur in any of these organs, while, in that of the ascites hepatoma very few tumors were formed there.

5) Lymph nodes—The increase of tumor cells are evident in both cases. But, in the case of the ascites hepatoma the enlargement of the metastasized lymph nodes is far more striking than that of the Yoshida sarcoma.

6) In the case of the ascites hepatoma, tumor formations in the lungs after the transplantation into the left chamber of the heart differ conspicuously from those after the transplantation into the right chamber of the heart (or the femoral vein), while there exists no such difference in the cases of the Yoshida sarcoma.

(B) Common points of both tumors

1) In both cases evident tumors occur frequently in the heart, adrenals, ovaries and pancreas.

2) Tumor formation was very rare in both cases in the spleen, thyroid glands, skeletal muscles, sublingual glands and prostate.

3) In the epididymis, parotid glands, submaxillary glands, tongue, aorta and bladder no increase of tumor cells was surely recognized in both cases.

Summary

1) The tumor ascites containing a large amount of free tumor cells of the Yoshida sarcoma (29 cases) and of the ascites hepatoma (22 cases) were transplanted into the left chamber of the heart of the rat, and the the state of formation of tumors in various organs was studied. Then a comparison of both tumors was made.

2) The transplantation of more than 300,000,000 tumor cells of the Yoshida sarcoma causes the death of animals transplanted in few minutes after the injection, but in such cases the tumor cells were evenly distributed in all organs examined.

3) The animals can survive the transplantation of about 10,000,000 cells (about 0.05 cc ascitic fluid) of the Yoshida sarcoma into the left chamber of the heart: the state of the tumor formation in various organs was investigated in such animals. In the case of the ascites hepatoma similar amount of ascitic fluid was transplanted and the organs were examined in the same way.

4) In the case of the Yoshida sarcoma conspicuous tumor formations in 6 to 20 days after the transplantation in such organs as the kidney, bone marrow, liver, stomach, intestine and thymus gland may be emphasized in comparison with that of the ascites hepatoma which is characterized by remarkable tumor increase in the brain and eye-ball. In the lymph nodes, heart, ovary, adrenal and pancreas evident tumor formation was noted in both tumors.

5) It was evidently demonstrated in this experiment that the rapidity of the tumor growth differed remarkably according to the various conditions of various organs, though the tumor cells were mechanically evenly distributed to every part of the body by the way of the arterial blood stream. The conditions may not be explained only by mechanical factors because of the predilecting organs of the sarcoma and the carcinoma as described above. But, at the same time, the experience may be remembered, that the tumors, when transplanted directly into any place, they grow at the site of the transplantation.

6) In these experiments, especially in that of the ascites hepatoma wide-spread hematogenous metastases of the lymph nodes were demonstrated. It may be considered that this fact affords one of the significant materials with which to explain the retrograde lymphogenous metastasis.

7) The above-mentioned findings were obtained by the experiments of the transplantation into the left chamber of the heart. In some cases the transplantation was made into the right chamber of the heart or into the femoral vein. The findings were as follows.

In the case of the ascites hepatoma almost all tumor cells were trapped in the capillaries of the lungs, and a significant tumor formation in the lungs arose, but few or no tumors in any other organ. However, in the case of the Yoshida sarcoma there occurred no significant difference. This may be due to the fact that in the case of the Yoshida sarcoma free tumor cells could easily pass through the capillaries of the lungs into the systematic arteries.

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Explanation of Plate XXV

Figure 1. A. A hemorrhagic metastasis of the stomach.
B. Bead-like tumors of the duodenum following the injection of the Yoshida sarcoma cells into the left chamber of the heart.

Figure 2. Enlargements of both ovaries and bead-like metastases in the uterus following the injection of the Yoshida sarcoma cells into the left chamber of the heart.

Figure 3. Tumor emboli in the capillary of the heart directly after the multitudinous (tumor ascites 1 cc) transplantation of the Ascites Hepatoma cells into the left chamber of the heart (high power).

Figure 4. Tumor metastases in the plexus chorioideus and the parenchyma of the brain following the injection of the Ascites Hepatoma into the left chamber of the heart.

Explanation of Plate XXVI

Figure 5. Microscopical finding of metastasis in the eye following the injection of the Ascites Hepatoma into the left chamber of the heart (low power).

Figure 6. Macroscopical finding of the same eye.

Figure 7. Tumor emboli in the arteriole near the glomerulus within 1 minute after the injection of the Ascites Hepatoma into the left chamber of the heart (high power).

Figure 8. Lungs of a rat following the injection of the Ascites Hepatoma into the right chamber of the heart.

Figure 9. Viscera of a rat following the injection of the Ascites Hepatoma into the left chamber of the heart.
Nodules of tumor are seen in the heart, right lung, both adrenals, right kidney, both ovaries, and mediastinal and retroperitoneal lymph nodes.

Explanation of Plate XXVII

Figure 10. "Rachitic" rosary-like tumor formation of ribs following the injection of the Ascites Hepatoma into the left chamber of the heart.

Figure 11. Skeletal muscles of a rat showing massive infiltration of tumors following the injection of the Ascites Hepatoma into the left chamber of the heart.

Figure 12. Hemorrhagic metastases of the Ascites Hepatoma in the skin in the same case as Fig. 11.

Figure 13. A high degree of metastasis in the retroperitoneal lymph nodes and also in the adrenal following the injection of the Ascites Hepatoma into the left chamber of the heart.
The kidney is not affected (low power).
A. Kidney B. Adrenal

要　旨

左側心臓内移植による全身諸臓器の腫瘍分布について
(吉田肉腫並びに腹水肝癌による研究)

神　崎　一　吉
(東北大学病理学教室，指導吉田富三教授)

1)　吉田肉腫(29例)及び腹水肝癌(22例)のシロネズミ左側心臓内移植実験を行い，各種臓器の腫瘍形成状態をおのおのの場合において検索し，さらに両者の比較を行った。

2)　いずれの腫瘍においても極めて多数の腫瘍細胞(吉田肉腫の場合では3億以上の数。注射後数分以内に動物が死亡する程の量)を左側心臓内に注入した場合には，検査した限りのすべての臓器において腫瘍細胞が満遍なく散布されていることが確認できる。

3)　吉田肉腫，腹水肝癌のいずれの場合においても細胞の純培養状態の腫瘍腹水約 0.05 cc (吉田肉腫の場合には細胞数約 10,000,000) の移植では動物は急死することなく腫瘍死を遂げる。平均生存日数は前者では 10 日(6～20日)，後者では 13日(8～22日)。かかる例について各種臓器の腫瘍形成状態が検索された。

4)　吉田肉腫では腫瘍死動物の剖検において腫瘍形成の特に顕著な臓器は腎，骨髄，肝，胃，腸及び胸腺であるのに対して，腹水肝癌においては脳，及び眼球における腫瘍形成が特に顕著である。淋巴腺，心，卵巣，副腎及び膵では両者のいずれの場合においても腫瘍形成が著しい。

5)　動脈血によって全身に平等に腫瘍細胞を散布させてしかも臓器により腫瘍成長の速度に著しく差違があることがこの実験において明かにされた。ことに肉腫と癌腫とにおいて腫瘍好発臓器が上述の如く明かに異ることは，これらの差違が単に機械的理由によってのみ説明出来ないことを示すものであろう。しかし，腫瘍はもし直接移植された場合には，その移植されたいずれの場所においても成長するということも同時に記憶されねばならないだろう。

6)　この実験において特に腹水肝癌の場合に，淋巴腺転移は血行によって強く起ってくるものであることが示された。このことは逆行性の淋巴行性の転移等の解釈に対して有意義な資料であると考える。

7)　以上は左側心臓内に移植して得られた所見であるが，右側心臓内または股静脈内に移植した場合には次の通りである。

腹水肝癌の場合には細胞はことごとく肺毛細管に捕捉されて，肺には極めて高度の腫瘍増殖

を起すが，肺以外の臓器には腫瘍の形成が極めて乏しいか，あるいは全く起らない。これに反して吉田肉腫の場合には左側心臓内移植の場合に比して著明な差を示さない。これは吉田肉腫の場合には個々の自由細胞が容易に肺の毛細管を通過し得るためであろう。

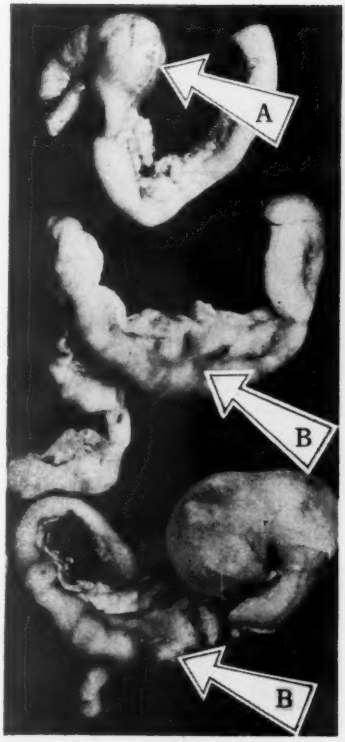

Fig. 1

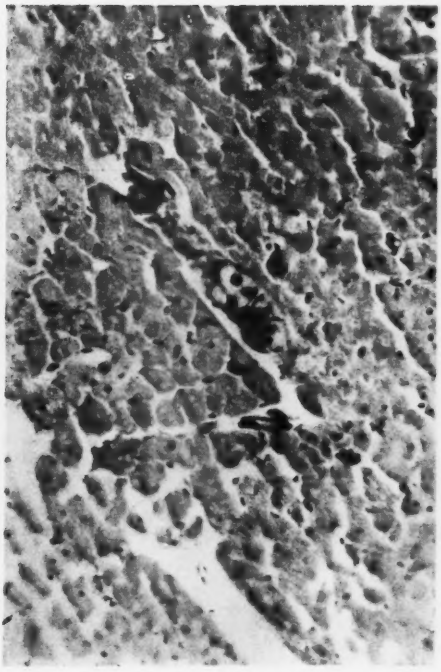

Fig. 3

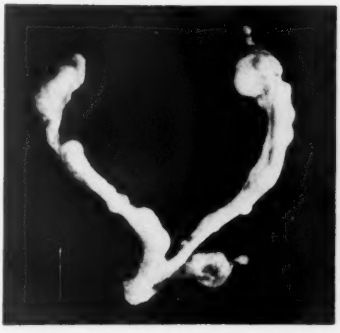

Fig. 2

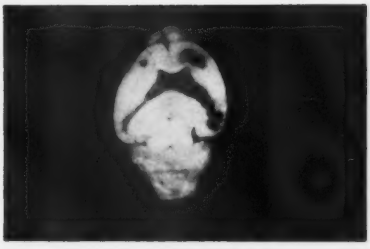

Fig. 4

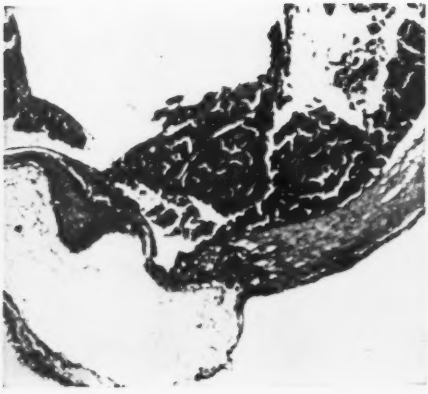

Fig. 5

Fig. 6

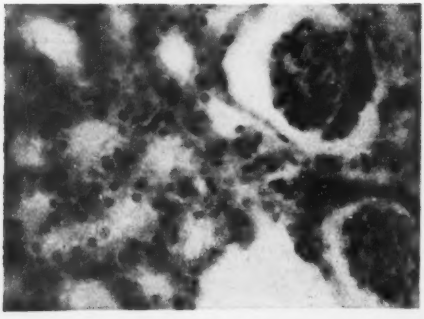

Fig. 7

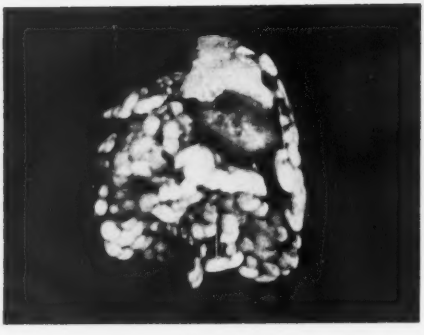

Fig. 8

Fig. 9

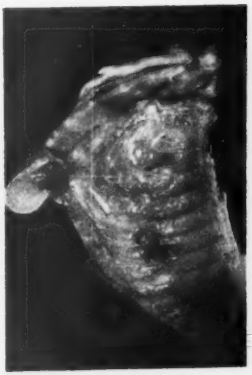

Fig. 10

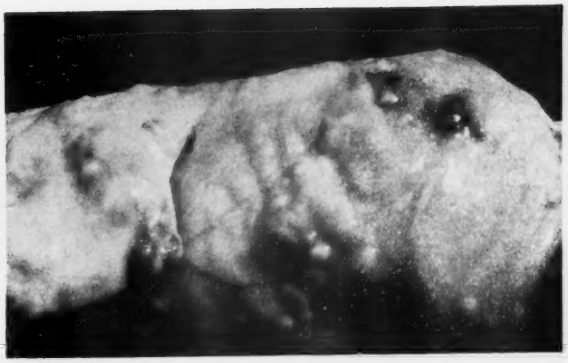

Fig. 11

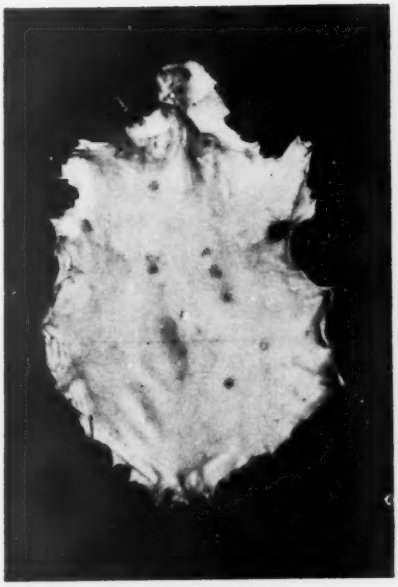

Fig. 12

Fig. 13

核　　酸

名大教授 理学博士　江上不二夫・柴谷篤弘著　　A5判 280頁 上製 定価 580円 〒40円

核酸そのものは有機化学者，生化学者，高分子物理化学者等により研究され，核酸の機能や代謝は生化学，細胞学遺伝学，細菌学，ヴィールス学，病理学を始め，生物学，医学のあらゆる分野で問題にされ重要視されている。本書はこの核酸について，驚くべき速さで広められ，深められている最近までの知見を収め詳らかに論述した。

主要目次　核酸研究の重要性・核酸，核蛋白の抽出，精製法・核酸の化学構造・核酸，核蛋白質の大きさと物理化学的諸性質・核酸の生化学・核酸の細胞化学・核酸の生物学的機能・ガンと核酸・索引

放射性追跡子実験技術

シュビツア，ホイットニー著　清水栄校閲　三輪博秀訳　　A5判 224頁 上製 定価 500円 〒40円

放射性追跡子を利用する実験室での研究及び指導の手引書として役立つように，最近数年間の長足の進歩をとりあげ放射性物質の取扱い注意，研究技術よりさらに各種実験を9章にわたつて詳密丁寧に解説した。また附録には一般的な知識となるものをあげた。入門書として関係学生から研究者・技術者は必読せねばならぬ好参考書。

主要目次　放射能の危険性・放射性実験室の運営・放射性実験室の構造・放射性実験室内での作業・基礎実験・化学実験・物理実験・生物学的実験・特殊試料調整法・実験に先立ちまたは実験と同時に行う講義，課程の概要

pH　概　説（共立全書）

東京大学講師　古賀正三著　　B6判 248頁 上製 定価 380円 〒30円

pH の理解に必要な溶液論・電気化学の基礎に始まつて，各種測定法を比較し，とくにガラス電極法に関しては詳述した。電気測定法・電子回路のことも最新のデータを使つて初歩から詳述してある関係学生・研究者向好入門書。

主要目次　水・溶液の熱力学と統計力学・電極の熱力学・弱電解質の理論・強電解質の理論・pH の定義・緩衝液・pH 指示薬・指示薬法による pH 測定・電気的測定のための準備・水素電極による pH 測定・キンヒドロンによる測定法・アニチモン電極法による測定・ガラス電極・ガラス電極による測定法・ガラス電極の応用例・pH に関係のある諸現象

系統的有機定性分析　純粋物編

熊本大学教授 理学博士　藤田穆著　　A5判 484頁 函入 定価 900円 送料40円

最もよく整理された分析書として，本書のコースを忠実に辿ることにより未知有機物を容易に判別し得るようにたもので，本書により今まで困難であった未知有機物検索が学生にも十分理解できるようになった斯界画期的試みの

主要目次　有機化合物諸性状の観測と構造への連関・性状及び構造の概括的予見・純粋有機化合物の分析系統・C.H.O よりなる化合物・C.H.O.N よりなる化合物・C.H.O.N 以外にさらに特殊元素ハロゲンを含有するもの・C.H.O.N.X 以外にさらに S を含有するもの・C.H.O.N.X.S 以外にさらに他の特殊元素を含有するもの・融点表

写　真　技　術（共立全書）

京都大学助教授　藤波重次著　　B6判 230頁 上製 定価 280円 送料 30円

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主要目次　緒論・乾板，フイルム・撮影・暗室設備・薬品と調剤・現像・定着，水洗，乾燥，保存・引伸と密着・焼付・迅速仕上法・幻燈写真(スライド)・カメラとレンズ・閃光撮影・望遠写真・解像力・写真像の異常効果・天然色写真・顕微鏡と電子顕微鏡写真・赤外線，紫外線写真・スペクトル写真・複写・製品一覧表・索引

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